Bioorganic & Medicinal Chemistry 22 (2014) 505–512

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Lipoic acid analogs with enhanced pharmacological activity ☆

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ARTICLE INFO

Article history:
Received 19 September 2013
Revised 23 October 2013
Accepted 31 October 2013
Available online 14 November 2013

Keywords:
Lipoic acid
Ischemia-reperfusion
SAR studies
Polymerization
Amino acids

ABSTRACT

Lipoic acid (1,2-dithiolane-3-pentanoic acid) is a pharmacophore with unique antioxidant and cytoprotective properties. We synthesized a library based upon the condensation of natural and unnatural amino acids with the carboxylic acid moiety of lipoic acid. SAR studies were conducted using a cardiac ischemia-reperfusion animal model. Cytoprotective efficacy was associated with the R-enantiomer of the dithiolane. Potency of library compounds was dictated by the acidic strength of the adduct. α-*N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine, designated CMX-2043, was chosen for further pharmacologic evaluation.

1. Introduction

Pharmacological protection against cellular damage and apoptotic death is a serious clinical need.[1] Although a large number of candidate molecules have been evaluated for this property in animal studies and human trials,[2,3] none has yet attained significant clinical usefulness. One such molecule is α-lipoic acid (LA), known primarily as a naturally occurring cellular antioxidant. Its activity in both aqueous and lipophilic, and intracellular or extracellular environments, both in oxidized and reduced forms, suggests potential opportunities for broad pharmacological action.[4]

Lipoic acid is naturally present in both prokaryotic and eukaryotic cells. It is recognized as a complement of α-keto acid dehydrogenase complexes of mitochondria and thus fundamental mammalian metabolism.[5] Due to its water and lipid soluble properties, exogenously administered LA is distributed in cellular membranes, cytosol and extracellular spaces.[6] Although considered as an antioxidant,[7–10] LA has regulatory action on signal transduction processes involved in tissue damage and protection[11] including activation of AKT phosphorylation.[6] Following an oral dose it is excreted largely by the kidneys.[12] It is metabolized extensively in the liver, including a high first-pass effect.[13] LA has been shown to be efficacious for prevention or treatment of ischemic injury to liver,[14–17] kidney,[18–21] heart,[6,22–26] and brain.[27–29] It is available in Germany in oral and parenteral formulations.[30,31]

The purpose of the present study was to prepare and screen a selection of LA analogs for cytoprotective activity. LA is an attractive candidate for modification based on both its chemical and biological properties. Structure activity relationships were investigated by assessing pharmacological efficacy of the analogs in a standard ischemia-reperfusion injury animal model.

2. Results and discussion

The molecular design adopted for these studies was an outgrowth of earlier work showing pharmacological activity of the fatty acid DHA (docosahexanoic acid) linked to a dodecapeptide.[32] This earlier molecule included an amide bond to an initial Asp-Gly sequence. In the present work the fatty acid was lipoic acid and the peptide sequence was shortened.[33,34] Cardiac ischemia-reperfusion injury was chosen to define structure–activity relationships.

2.1. Screening assay

In a multi-factorial condition such as ischemia-reperfusion injury (IRI) with complex contributory mechanisms, approaches based upon traditional receptor-based drug design have not succeeded clinically.[35] It has been recently recommended that the preclinical testing of potential cardioprotective agents should employ clinical trial-like approaches.[36] Since the goal of our development program was to develop an agent effective for IRI, an unconventional screening approach was selected based upon efficacy in an animal model of cardiac injury. Although labor intensive, this

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http://dx.doi.org/10.1016/j.bmc.2013.10.057

approach simultaneously addressed issues related to ADME ('drugability') and toxicity within the single efficacy measure. Efficacy was measured in a widely used rat model of myocardial ischemia-reperfusion injury (IRI).[37,38] Male Sprague Dawley rats (6–12/group dependent on the experiment) were used in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, The National Academies Press Eighth Edition, 2011). Animal studies were approved by the Ischemix Animal Care and Use Committee. In this model, following anesthesia, thoracotomy, and vertical pericardotomy, the left circumflex (LCX) coronary artery was ligated for 30 min to induce ischemia. During the ischemic period, fluorescent microspheres were injected through the apex of the heart into the left ventricular cavity to delineate the area at risk (AR) by the absence of fluorescence. Following the ligation period, the heart was allowed to reperfuse and the animals to recover. After 24 h, the animals were sacrificed and the hearts excised and sliced transversely into sections 2 mm thick. The slices were stained to distinguish live tissue from the unstained myocardial infarct (MI) area. Test animals were administered a single injection of test agent into the left ventricular cavity prior to LCX ligation. The AR and MI areas were quantitated to obtain the MI/AR ratio. More efficacious compounds have smaller MI/AR ratios.

2.2. Peptide and stereoisomer selection

In initial studies, the efficacy of peptide sequences capped with an acetyl group was evaluated. The Asp-Gly sequence in the dodecapeptide was replaced with the more chemically stable dipeptide Glu-Ala (EA) to avoid known aspartimide formation.[39] These studies showed that the di- and tetrapeptide Glu-Ala motifs had greater efficacy than that of a heptapeptide (Table 1). Based on this finding, the shorter dipeptide sequence Glu-Ala was selected for attachment to lipoic acid to undergo further evaluation.

LA occurs naturally as the R-enantiomer and acts as a coenzyme in many reactions. It constitutes a growth factor for a number of bacteria and protozoa. In studies with synthetic lipoic acid the R-isomer has been described as the more active form as well as the enantiomer that is more readily absorbed following oral administration (38% for the R-form vs 28% for the S-form in humans).[40,41] The R-enantiomer has primarily an anti-inflammatory activity while the S-enantiomer has an antinociceptive (analgesic) activity. In addition to these properties, both R- and S-enantiomers possess cytoprotective activity. Wolz and Krieglstein describe that the S-enantiomer of lipoic acid had a stronger neuroprotective effect than the R-enantiomer in a mouse model of cerebral ischemia.[42] In view of the demonstrated variability in effects of lipoic acid stereospecificity, the two stereoisomers of Lip-EA-OH were tested to determine their relative efficacies in the rat IRI model (Table 2).

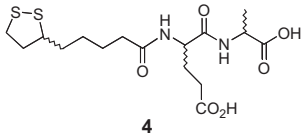
Table 1
MI/AR reduction of lead sequence

Entry	Peptide sequence	MI/AR reduction (%)
1	Ac-EAEAEGA-OH	17 ($p < 0.01$)
2	Ac-EAEA-OH	23 ($p < 0.05$)
3	Ac-EA-OH	23 ($p < 0.05$)

Table 2
Stereoisomers of Lip-Glu-Ala-OH

Entry	Compound name	Optical rotation (EtOH, 25 °C, 10 mg/mL)
4a	(R)-Lip-ʟGlu-ʟAla-OH	+36.1
4b	(S)-Lip-ʟGlu-ʟAla-OH	−69.5
4c	(R/S)-Lip-ʟGlu-ʟAla-OH	−24.2

The ***N***-terminal amino function of the peptide H_2N-ʟGlu-ʟAla-OH (**5**) was attached to the carboxylic acid of either the R- or S-stereoisomers, as well as the R/S racemic mixture, to provide **4a**, **4b**, and **4c**, respectively. The optical activity of **4a**, **4b**, and **4c** is +36.1, −69.5, and −24.2, respectively (Table 2). A single IC bolus of **4a** at doses of 1 and 2 mg/kg provided an MI/AR ratio of 0.33 and 0.29, respectively, correlating to a MI/AR reduction of ~21%. A single IC bolus of **4b** at doses of 1 and 2 mg/kg provided an MI/AR ratio of 0.40 correlating to a MI/AR reduction of ~10%. A single IC bolus of **4c** at a dose of 2 mg/kg provided an MI/AR ratio of 0.33 correlating to a MI/AR reduction of ~21%. Finally, a dose of a mixture **4a** (1 mg/kg) and **4b** (1 mg/kg) provided an MI/AR ratio of 0.33 correlating to an MI/AR reduction of 21%. These results clearly demonstrate that the R-enantiomer of LA (**4a**) provides more efficacy than its corresponding S-isomer, and the former was chosen as the lead candidate for further investigation.



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2.3. Chemistry

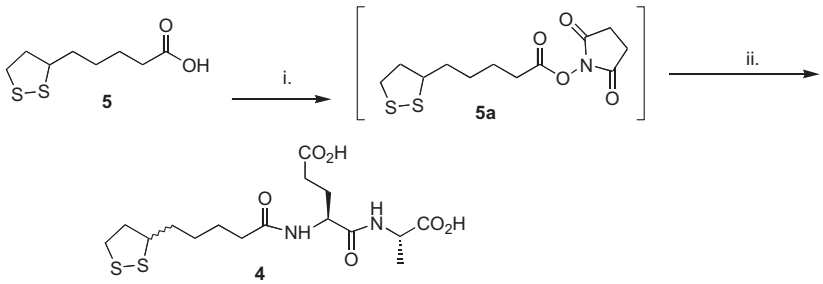
Since it is known that lipoic acid can polymerize, this possibility had to be taken into consideration in synthesis of the planned analogs. Photo instability of lipoic acid was described by Barltrop et al. in 1954[43] and Wagner et al. in 1956.[44] Its degradation in the presence of light was characterized by a physical change in the compound and a shift in the ultraviolet spectrum. The instability was proposed to be due to the polymerization of lipoic acid via opening of the strained dithiolane ring followed by formation of intermolecular disulfide linkages.[45]

The effects of solvent on lipoic acid photolysis and polymerization have also been evaluated.[46] Brown and Edwards found that solvents with readily extractable hydrogen inhibited light induced polymerization of lipoic acid and reported that this tendency was minimized in the presence of 2-propanol. This phenomenon is consistent of a mechanism of lipoic acid polymerization via a photolytic opening of the dithiolane ring structure resulting in a diradical, followed by propagation through intermolecular disulfide bond formation.

Efforts to produce lipoic acid free from contaminating polymer have focused on crystallization solvents[47,48] and salt formation.[49–51] Beisswenger et al. crystallized lipoic acid at low temperature from mixtures of solvents selected from pentane, cyclohexane, ethyl acetate and ethers.[48] Klatt et al. crystallized pharmaceutical grade lipoic acid at low temperature from organic solvents with a dielectric constant between 1.95 and 2.4, such as hexane or heptane.[47] Hettche, Rischer, and Sarlikiotis prepared sodium and tromethane (tris) salts and drug product formulations containing mixed and single isomers of lipoic acid.[49] Ames stabilized lipoic acid via the production of a nicotinamide salt.[50]

Majeed and Nagabhushanam prepared an adduct of lipoic acid consisting of the *N*',*N*'-(dimethylamino)ethylamino amide.[51] This compound was crystallized as a stable nonhydroscopic fumarate or maleate salt, although propensity toward polymerization was not directly addressed. The potential of other lipoic acid derivatives to polymerize has not been generally addressed in the synthetic literature.[33]

To prepare **4**, α-lipoic acid was added to a solution of *N*,*N*-diisopropylethylamine (DIPEA) in acetone and treated with *N*,*N*'-disuccinimidyl carbonate (DSC) (Scheme 1). After CO_2 evolution, the resulting succinimidyl ester solution **5a** was cooled and treated



Scheme 1. Reagents and conditions: (i) DSC, DIPEA/acetone. (ii) Glutamyl-alanine, NaOH/acetone/water.

rapidly with an aqueous NaOH solution of glutamyl-alanine. After the reaction was complete, workup involved removal of acetone under vacuum, addition of water and ethyl acetate, and acidification with aqueous HCl. For small scale reactions (<1 g), purification of **4** was accomplished via preparative HPLC. For initial large-scale preparations (>100 g), the final step in the synthetic process was crystallization from ethyl acetate and *n*-butanol.

2.4. Polymerization

During the initial large-scale preparations of **4c**, and subsequently **4a**, analysis of the isolated product via HPLC indicated a peak corresponding to **4** and a broad later eluting peak (Fig. 1). The late eluting impurity was observed upon isolation of the solid product. The mother liquor from the crystallization was analyzed by HPLC and did not contain the impurity. Therefore, the impurity was formed during crystallization or precipitation of **4**. The impurity was detected and characterized as intermolecular linked disulfide polymers.

A size exclusion chromatographic (SEC) HPLC method was developed to detect potential polymer contamination in **4** using a Tosoh Bioscience TSK guard column PW_{XL} (6 mm × 4 cm) with a Tosoh Bioscience TSK-Gel $PW2500_{XL}$ (7.8 mm × 30 cm) column. This method was able to separate analytes based on molecular weight and it was determined that the later eluting impurity in Figure 1 was a higher molecular weight moiety. Furthermore, an approximation of the molecular weight impurity size was determined using molecular weight cutoff spin cartridges (Micron filter device Ultracel YM-3 and YM-10). The material retained on the YM-10 filter (regenerated cellulose 10,000 molecular weight cutoff) was predominantly the late eluting impurity.

To determine if the impurity in the crystallized product was a disulfide linked polymer of **4**, the mixture was treated with a reducing agent. The product containing both **4** and the impurity was treated with dithiothreitol (DTT) and the mixture was analyzed by HPLC. Analysis of the HPLC chromatogram indicated the disappearance of the broad, late eluting impurity peak and the peak corresponding to **4** was substantially diminished. Analysis of the HPLC chromatogram also indicated the appearance of a peak corresponding to the disulfhydryl analog of **4** containing the dihydrolipoyl moiety. DTT treatment reduced both the dithiolane in **4** and the disulfide bridges of the polymeric material to a single product corresponding to the dithiol analog of **4**. Therefore, the impurity observed in **4** was determined to be the intermoleclular disulfide polymer of the lipoyl function in **4**.

To avoid the formation of the intermoleclular disulfide polymer during large scale preparation, a secondary alcohol such as 2-propanol has been reported to inhibit the polymerization of lipoic acid.[46] Crystallization conditions were changed to isopropyl acetate–2-propanol from the original solvent mixture of ethyl acetate–*n*-butanol. In addition, steps were taken to avoid reducing the solvent mixture to a low volume and inducing crystallization.

2.5. Chiral purity

Efforts to resolve the diastereomers of **4c** were undertaken. We observed that **4a** and **4b** do not resolve via reversed-phase HPLC and chiral chromatography. Alternatively, **4d** in which L-Ala is replaced with D-Ala is readily resolved from **4a** and **4b** by reversed-phase HPLC on a C_{18} column.

A survey of the literature provided a procedure for resolution of the enantiomers of lipoic acid.[52] Treatment of lipoic acid with a reducing agent followed by reaction with *o*-phthalaldehyde and L-phenylalanine provides lipoic acid derivatives that are resolved by HPLC. An investigation to determine if this procedure could be applied to the resolution of **4c** was performed (Boston Analytical). Compounds **4a**, **4b**, and **4c** were treated with a reducing agent 2followed by reaction with *o*-phthalaldehyde and L-phenylalanine. The HPLC analysis method for the derivatives of **4a**, **4b**, and **4c** was not specific due to the co-elution of side products from the

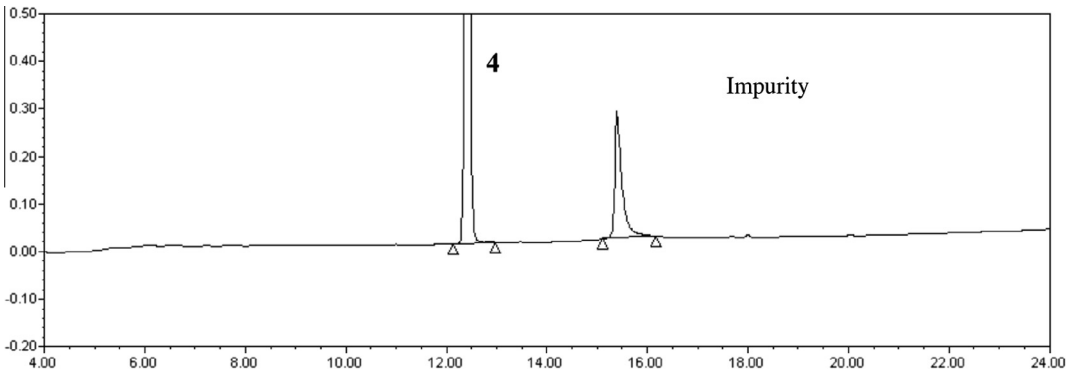


Figure 1. HPLC analysis of preparation of **4**.

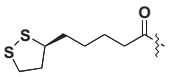
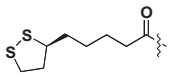
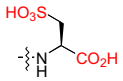
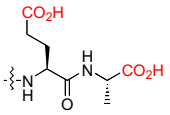
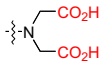
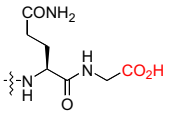
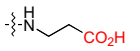
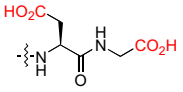
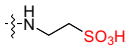
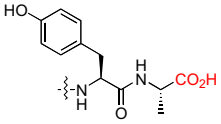
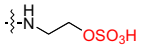
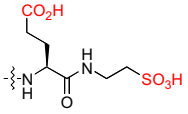
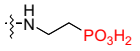
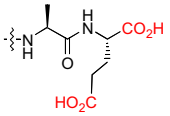
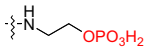
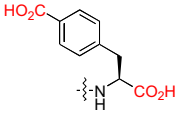
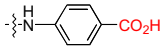
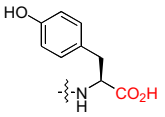
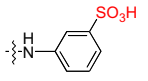
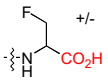
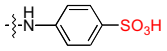
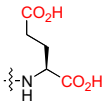
derivatization procedure and thus the chiral purity of **4a**, **4b**, and **4c** could not be established. Due to the distance between the chiral centers of the lipoyl and glutamyl moieties, chiral resolution of the diastereomers of **4c** and corresponding assay for purity of the single stereoisomer of **4a** is a serious challenge and will be explored further.

Based upon the enhanced biological efficacy of **4a** compared to **4b**, the development program focused on the preparation of α-lipoic acid adducts incorporating the R-enantiomer. A small library of compounds was prepared to explore further the role of the acidic strength. Both alkyl and aryl carboxylic, sulfonic and phosphonic derivatives were synthesized using the procedure outlined above. α-Lipoic acid adducts were produced by first in situ activation of the carboxylic moiety of lipoic acid with disuccinimidyl carbonate (DSC) in the presence of a tertiary amine in an acetone solution followed by the condensation of the activated lipoic acid with a commercially available amino-containing compound in a semi-aqueous solution. Polymer-free compounds were isolated from the crude reaction mixture either by preparative HPLC or after extractive removal of reactants by crystallization from a solution of isopropyl or ethyl acetate containing 2-propanol and water. Structure confirmation and purity was obtained by HPLC, mass spectrometry and ^{1}H NMR spectroscopy.

2.6. Structure activity relationships

The results of the cytoprotection assay for the various LA analogs are presented in Table 3. Test compounds were dissolved in isotonic saline at a dose volume of 1 mL/kg and were administered

Table 3
MI/AR reduction and pK_a values of lipoic acid adducts

Compound number		% MI/AR reduction (p value)**	pK_a*	Compound number		% MI/AR reduction (p value)**	pK_a*
5		19 ($p > 0.05$)	4.52	**15**		30 ($p < 0.05$)	−1.28
4a		31 ($p < 0.01$)	3.50	**16**		21 ($p > 0.05$)	3.31
6		17 ($p > 0.05$)	3.73	**17**		39 ($p < 0.01$)	4.45
7		28 ($p < 0.05$)	3.38	**18**		45–52 ($p < 0.001$)	−1.01
8		31 ($p < 0.05$)	3.73	**19**		30 ($p < 0.05$)	−1.74
9		33 ($p < 0.01$)	−1.22	**20**		53 ($p < 0.001$)	1.79
10		24 ($p < 0.05$)	3.52	**21**		47 ($p < 0.01$)	1.54
11		26 ($p < 0.05$)	3.39	**22**		22 ($p > 0.05$)	4.16
12		29 ($p < 0.05$)	3.71	**23**		33 ($p < 0.05$)	−2.05
13		10 ($p > 0.05$)	3.89	**24**		44 ($p < 0.01$)	−2.16
14		33 ($p < 0.01$)	3.52				

* Theoretical calculation using MarvinSketch Version 5.5.0.1 with pK_a plugin.
** p Values paired against placebo.

as a single intravenous bolus 15 min prior to the 30 min ligation at doses between 1–10 mg/kg. Specifically, the free acid of **4a** was added to a solution that included sufficient 0.5 N NaOH for its neutralization, NaCl, and water in quantities calculated to produce 285 mOsm, that is, physiological osmolarity. Prior to efficacy testing, formulated solutions were confirmed for both **4a** concentration and absence of polymer by analysis of the HPLC chromatogram. The MI/AR ratio was calculated and the reduction of test compound MI/AR to that for the vehicle was calculated. The parent α-lipoic acid yielded only a 19% MI/AR reduction in this model.

To explore the nature of the dipeptide adduct of **4a**, **7** was prepared in which one methylene group was removed in each side-chain (L-Asp for L-Glu; Gly for L-Ala). Similar efficacy to **4a** (28% MI/AR reduction compared to placebo) was achieved indicating that the methylene tether is not critical. In addition, inverting the dipeptide sequence to L-Ala-L-Glu in **10** also provided good efficacy (24% MI/AR reduction compared to placebo). However, removal of the acidic side-chain in **4a** and replacement with a carboxamide (**6**) eliminated efficacy. This result corroborates in vitro data demonstrating the requirement of a carboxylic acid function, although the location of the acidic residue is not critical. The phenolic function of the side-chain of a tyrosine residue in **8** provides enough acidity to provide efficacy (31% MI/AR reduction compared to placebo). Lastly, to explore the role of the C-terminal acidic function, L-Ala was replaced with taurine in **9** to incorporate the stronger sulfonic acid function. The sulfonic acid residue provided slightly better efficacy than its corresponding carboxylic acid (33% vs 31%).

To determine the role of the alkyl side chain of L-Ala, **14** was prepared in which the residue was removed. Slightly improved efficacy was achieved compared to **4a** which incorporates L-Ala (33% vs 31%). Replacement of the carboxylic acid function of Glu with a sulfonic acid (cysteic acid [L-Cya]) **15**, phenol (L-Tyr) **12**, and aryl carboxylic acid (4-carboxy-L-Phe) **11** provided compounds with similar efficacy (30%, 29%, and 26%, respectively). Minimal efficacy was achieved when 3-fluoro-D/L-alanine (10% MI/AR reduction compared to placebo). These results demonstrate the significance of the side-chain function. Potency is achieved when one side-chain residue contains an acidic function such as a carboxylic, sulfonic acid or phenol. Compounds **4a**, **7**, **8**, **9**, **10**, **11**, **12**, **14**, **15** contain 2 acidic functions derived from proteogenic and nonproteogenic amino acids. α-Lipoic acid was treated with iminodiacetic acid (Idaa) to form **16** containing a tertiary amide with a bis-carboxylic acid. Surprisingly, **16** provided a low level of efficacy (21% MI/AR reduction compared to placebo). Presumably the lower efficacy is attributed to the incorporation of a tertiary amide as opposed to other analogs that contain a secondary amide.

Linear alkyl adducts as opposed to α-amino acids were attached to α-lipoic acid and their efficacy was measured. βAla, taurine, aminoethyl hydrogen sulfate, phosphorylethanolamine, and aminoethylphosphonic acid were condensed with activated α-lipoic acid to provide **17**, **18**, **19**, **20**, and **21**, respectively. The MI/AR reduction compared to placebo for **17**, **18**, **19**, and **20** was 39%, 45–52%, 30%, 53%, and 47%, respectively. The linear alkyl adducts provided the highest level of efficacy. Presumably this effect was due to both the incorporation of a linear alkyl chain and more importantly acid functions with stronger pK_a values.

The corresponding aryl carboxylic and sulfonic acids **22**, **23**, and **24** were prepared and their efficacy was determined. The MI/AR reduction compared to placebo for **22**, **23**, and **24** was 22%, 33%, and 44%, respectively. These results clearly demonstrate that an alkyl acidic function is more favored than an aryl function (**14** vs **11**, **17** vs **22** and **18** vs **23**, **24**). Aryl substitution is favored in the 4-position (**24**) as opposed to the 3-position **23**.

Interestingly, adducts to the lipoyl moiety containing 2 acidic functions generally were less efficacious than a similar adduct with only one acidic function. **14** (R-Lip-L-Glu-OH) containing a side-chain carboxylic acid was less efficacious (33%) than **17** (R-Lip-βAla-OH) (39%); **9** (R-Lip-L-Glu-Tau) containing a carboxylic acid side-chain and terminal sulfonic acid and **15** (R-Lip-L-Cya-OH) containing a side-chain sulfonic acid were less efficacious (33% and 30%, respectively) than both **17** and **18** R-Lip-Tau-OH) (45–52%). The theoretical pK_a values for lipoic acid analogs **5**–**24** were calculated using MarvinSketch Version 5.5.0.1 with pK_a plugin (Table 3). A scatter plot correlating the efficacy determined by reduction in MI/AR ratio compared to placebo as a function of theoretical pK_a values for lipoic acid analogs **5**–**24** is displayed in Figure 2. Analysis of the scatter plot indicates that modifications to α-lipoic acid with adducts that yield a final product with pK_a values between 3 and 4 do not provide compounds that were as efficacious based upon reduction of MI/AR ratio to placebo than analogs with pK_a values below 2. Interestingly, the optimal results were obtained with compounds that have a pK_a value between 1 and 2. Although optimization for dose administration for each compound was not performed, the data clearly indicates a trend that the highest level of efficacy should be achieved with α-lipoic acid analogs possessing a calculated pK_a values below 2.

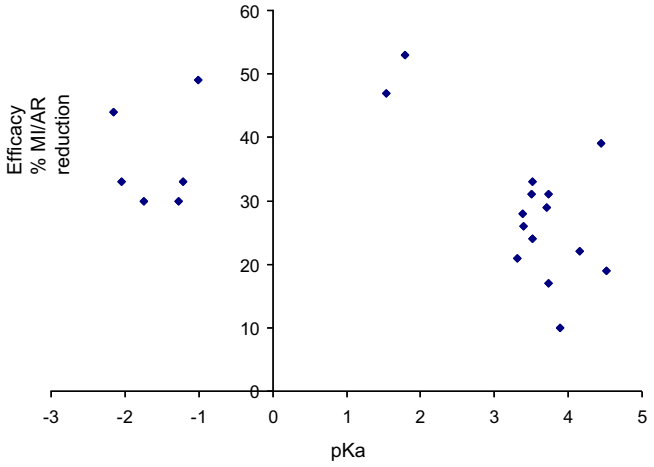


Figure 2. Scatter plot analysis of lipoic acid analogs efficacy as a function of pK_a.

3. Conclusion

A discrete library based upon condensation of natural and unnatural amino acids with the carboxylic acid moiety of LA was prepared and evaluated for cytoprotective activity. SAR studies in a cardiac IRI model indicated that potency was dictated by the acidic strength of the modification. Linear alkyl acidic (carboxylic, sulfonic, phosphoric) adducts to LA yielded the most efficacious compounds. Linear alkyl adducts were more effective than their corresponding aryl adducts. Analogs containing two acidic functions (carboxylic or sulfonic acid) were less efficacious than compounds modified with only one acidic function. Modifications that did not contain an acidic function were the least efficacious. From these studies, sulfonic acid/sulfuric acid > phosphonic acid/phosphoric acid > carboxylic acid in reducing of MI/AR ratio effects in a rat model of ischemia-reperfusion injury.

These studies support the potential utility of lipoic acid-peptide analogs in treating cellular ischemia-reperfusion injury in clinical settings. The choice of a clinical candidate involves more than biological efficacy in an animal model. This work also provided insights to the ease of synthesis and compound stability. The analog **4a** (*N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine) was selected based upon these attributes as well as a favorable ADMET

and receptor screen profile for further preclinical and clinical investigation.[53] In a human trial, **4a**, designated CMX-2043, showed statistically significant protection against myocardial damage associated with percutaneous coronary intervention (PCI).[54]

4. Material and methods

4.1. General experimental procedures

All reagents were purchased from commercial sources and used without further purification. Racemic lipoic acid was obtained from Sigma–Aldrich (US), R-lipoic acid from Labochim (Italy), and S-lipoic acid from Shanghai Freeman Lifescience (China). Natural and unnatural amino acids were obtained from either Sigma–Aldrich (US) or Bachem (Switzerland); dipeptides from Bachem, chemical reagents from Sigma–Aldirch, and solvents from VWR (US). Acetylated peptides **1**, **2**, and **3** were obtained either from CS Bio (San Carlos, CA) or MidWest Biotech (Fishers, IN). Optical rotation was performed with a Rudolph Polarimeter model Digi-Pol-781 SDV. RP-HPLC chromatography was performed with a Waters 1525 binary pump, 717 plus autosampler coupled to a dual band detector 2487. The analyses were conducted using a YMC-PackPro C_{18} column (100 × 4.6 mm I.D), a mobile phase linear gradient, and monitoring by UV absorption at 220 nm. Mass spectra were provided by HT Laboratories or Creagen. Identity of all final compounds was assessed by mass spectrometry.

4.2. Library synthesis

General procedure for the synthesis of α-lipoic acid analogs: (R)Lipoic acid (ʀLip-OH, 10.0 g, 48.5 mmol) was dissolved in acetone (100 mL, 10 mL/g). The solution was protected from direct light by covering the reaction flask with foil. *N,N*-Disuccinimidylcarbonate (15.5 g, 1.25 equiv, 60.6 mmol) and *N,N*-diisopropylethylamine (DIEA, 10.5 mL, 1.25 equiv, 60.6 mmol) were added sequentially and the reaction was stirred vented for 2 h at room temperature to form Lip-OSu in situ. The corresponding amines (1.15 equiv) were added to the solution of Lip-OSu in acetone, followed by the addition of water (50 mL) and DIEA (19.4 mL, 2.3 equiv, 112 mmol). The combined solution was stirred overnight. Approximately one third of the reaction mixture was then reduced to approximately half volume on a rotary evaporator. The remaining reaction mixture was injected multiple times directly onto a semi-preparative high-performance liquid chromatography (HPLC) system and the product isolated on a YMC Pack Pro C18 reverse phase column using a gradient of increasing acetonitrile (0.5% acetic acid) in water (0.5% acetic acid). Product-containing fractions were identified by analytical HPLC, frozen, and lyophilized.

4.2.1. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine (4a)

6.9 g (35%); HPLC: >99% (rt: 12.3 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.29 (br s, 2, OH), 8.19 (d, 1, J = 7.15, NH), 7.93 (d, 1, J = 7.93, NH), 4.30 (m, 1, CH), 4.17 (m, 1, CH), 3.60 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S), 2.45–2.35 (m, 1), 2.28–2.34 (m, 2), 2.18-2.08 (m, 2), 1.91–1.84 (m, 2), 1.75–1.62 (m, 2), 1.56–1.48 (m, 3), 1.36–1.32 (m, 2), 1.27 (d, 3, J = 7.25, CH_3); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 174.0 (CO_2H, CO_2H), 172.0 (CONH), 171.1 (CONH), 56.1 (NHCHCO), 51.3 (NHCHCO), 47.4, ($SCH(CH_2)_2$) 39.5 (SCH_2), 38.1 (CH_2), 34.9 (CH_2), 34.1(CH_2), 30.0(CH_2), 28.2(CH_2), 27.6(CH_2), 25.0 (CH_2), 17.0 (CH_3); mp 128–130 °C; IR (KBr pellet) 3281, 3300–2500, 1722, 1638, 1546, 1453, 1425, 1273 cm^{-1}; ESI-MS: m/z = 405.33 (M−1); Anal. Calcd for $C_{16}H_{26}N_2O_6S_2$: C, 47.3; H, 6.4; N, 6.9; S, 15.8. Found C, 46.8 H, 6.4; N, 6.9; S, 15.4; +36.1 (EtOH, 25 °C, 10 mg/mL).

4.2.2. *N*-[(S)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine (4b)

Prepared from S-lipoic acid. HPLC: >99% (rt: 12.3 min); −69.5 (EtOH, 25 °C, 10 mg/mL).

4.2.3. *N*-[(R/S)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine (4c)

Prepared from R/S-lipoic acid. HPLC: >99% (rt: 12.3 min); −24.2 (EtOH, 25 °C, 10 mg/mL).

4.2.4. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutaminyl-glycine (6)

HPLC: 100% (rt: 10.0 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.2 (d, 1, J = 7.15, NH), 7.9 (d, 1, J = 7.93, NH), 4.85 (m, 1, CH), 3.90 (d, 2H, CH), 3.10 (m, 1), 3.60 (m, 1, CH-S), 3.19-3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{15}H_{25}N_3O_5S_2$ = 392 (M−1).

4.2.5. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-aspartyl-glycine (7)

HPLC: >99% (rt: 9.19 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.2 (d, 1, J = 7.15, NH), 7.9 (d, 1, J = 7.93, NH), 4.30 (m, 1, CH), 4.17 (m, 1, CH), 3.60 (m, 1, CH-S), 3.19-3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{14}H_{22}N_2O_6S_2$ = 379 (M+1).

4.2.6. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-tyrosinyl-L-alanine (8)

HPLC: >98% (rt: 13.1 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.1 (d, 1, J = 7.15, NH), 8.0 (d, 1, J = 7.93, NH), 4.63 (m, 1, CH), 4.40 (m, 1, CH), 3.60 (m, 1, CH-S), 3.19-3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{20}H_{28}N_2O_5S_2$ = 439 (M−1).

4.2.7. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-taurine (9)

HPLC: 100% (rt: 9.05 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.3 (d, 1, J = 7.15, NH), 7.9 (d, 1, J = 7.93, NH), 4.20 (m, 1, CH), 3.36 (m, 1, 2H, CH_2), 3.60 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{15}H_{26}N_2O_7S_3$ = 441 (M−1).

4.2.8. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-alaninyl-L-glutamic acid (10)

HPLC: 98% (rt: 11.25 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.3 (d, 1, J = 7.15, NH), 7.9 (d, 1, J = 7.93, NH), 4.20 (m, 1, CH), 3.36 (m, 1, 2H, CH_2), 3.60 (m, 1, CH-S), 3.19-3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{16}H_{26}N_2O_6S_2$ = 405 (M−1).

4.2.9. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-(4-carboxy)-phenylalanine (11)

HPLC: 99% (rt: 12.69 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.9 (d, 1, J = 8.0, NH), 4.20 (m, 1, CH), 3.36 (m, 1, 2H, CH_2), 3.60 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{18}H_{23}NO_5S_2$ = 398 (M+1).

4.2.10. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-tyrosine (12)

HPLC: 95% (rt: 12.98 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.9 (d, 1, J = 8.0, NH), 4.20 (m, 1, CH), 3.60 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{17}H_{23}NO_4S_2$ = 370 (M+1).

4.2.11. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-D/L-β-flouro-alanine (13)

HPLC: 100% (rt: 12.49 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.1 (d, 1, J = 8.0, NH), 4.13 (m, 1, CH), 3.60 (m, 1, CH-S), 3.19-3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{11}H_{18}FNO_3S_2$ = 296 (M+1).

4.2.12. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamic acid (14)

HPLC: 95% (rt: 12.98 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.0 (d, 1, J = 8.0, NH), 4.37 (m, 1, CH), 3.60 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{13}H_{21}NO_5S_2$ = 334 (M−1).

4.2.13. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-cysteic acid (15)

HPLC: 97% (rt: 7.61 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.0 (d, 1, J = 8.0, NH), 4.55 (m, 1, CH), 3.75 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{11}H_{19}NO_6S_3$ = 360 (M+1).

4.2.14. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-iminodiacetic acid (16)

HPLC: 90% (rt: 11.40 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.2 (d, 1, J = 8.0, NH), 4.20 (d, 4H, CH_2), 3.75 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{12}H_{19}NO_5S_2$ = 320 (M−1).

4.2.15. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-β-alanine (17)

HPLC: 100% (rt: 10.95 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.2 (d, 1, J = 8.0, NH), 4.20 (d, 4H, CH_2), 3.75 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{11}H_{19}NO_3S_2$ = 278 (M+1).

4.2.16. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-taurine (18)

HPLC: 100% (rt: 8.29 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.1 (d, 1, J = 8.0, NH), 4.16 (t, 2H, J = 7.10 CH_2), 3.75 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S), 2.98 (t, 2H, J = 7.05); ESI-MS: m/z calcd for $C_{10}H_{19}NO_4S_3$ = 312 (M−1).

4.2.17. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-2-aminoethyl hydrogen sulfate (19)

HPLC: 100% (rt: 9.05 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.0 (d, 1, J = 8.0, NH), 3.65 (m, 2H, CH_2), 3.75 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S), 2.98 (t, 2H, J = 7.05); ESI-MS: m/z calcd for $C_{10}H_{19}NO_5S_3$ = 328 (M−1).

4.2.18. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-2-aminoethyl phosphonic acid (20)

HPLC: 98% (rt: 8.63 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.9 (d, 1, J = 8.0, NH), 3.75 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S), 2.05 (m, 2H, CH_2); ESI-MS: m/z calcd for $C_{10}H_{20}NO_4PS_2$ = 312 (M−1).

4.2.19. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-*O*-phosphoryl-ethanolamine (21)

HPLC: 89% (rt: 8.32 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.2 (d, 1, J = 8.0, NH), 3.84 (q, 2H, J = 7.0, CH_2) 3.75 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S),; ESI-MS: m/z calcd for $C_{10}H_{20}NO_5PS_2$ = 328 (M−1).

4.2.20. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-4-aminobenzoic acid (22)

HPLC: 99% (rt: 15.25 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.1 (d, 1, J = 8.0, NH), 3.75 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S),; ESI-MS: m/z calcd for $C_{15}H_{19}NO_3S_2$ = 326 (M+1).

4.2.21. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-3-aminobenzene sulfonic acid (23)

HPLC: 95% (rt: 12.50 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.1 (d, 1, J = 8.0, NH), 3.75 (m, 1, CH-S), 3.19–3.05 (m, 2, CH_2-S),; ESI-MS: m/z calcd for $C_{14}H_{19}NO_4S_3$ = 360 (M−1).

4.2.22. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-3-aminobenzene sulfonic acid (24)

HPLC: 92% (rt: 12.37 min); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.0 (d, 1, J = 8.0, NH), 3.75 (m, 1, CH-S), 3.19-3.05 (m, 2, CH_2-S); ESI-MS: m/z calcd for $C_{14}H_{19}NO_4S_3$ = 360 (M−1).

4.3. Biological study

Rat model of I-R injury. Male Sprague Dawley rats were used in accordance with the Guide for the Care and Use of Laboratory Animals. Animal studies were approved by the Ischemix Animal Care and Use Committee. A standard rat model of myocardial ischemia-reperfusion injury was used to evaluate the protective potential of lipoic acid analogs.[12,13] This model is analogous to the I/R injury observed in patients following coronary occlusions and cardiac surgery procedures, such as percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). Male Sprague Dawley rats (6–12/group dependent on the experiment) (Charles River) between 300 and 350 g were anesthetized with 3–4% isoflurane in an induction chamber. Anesthesia was maintained at a surgical plane with 1.5–2.0% isoflurane, administered by a rodent ventilator (Harvard Apparatus Inc.) through a 16-gauge endotracheal tube. The ventilator was set at 2.5 cc at a rate of 60–65 breaths per minute to maintain ventilation during surgery. The core temperature of the animal was monitored with a rectal probe and maintained at 37 °C with a heating lamp attached to a temperature controller (Physitemp Instruments Inc).

A left anterior thoracotomy was performed and the heart was exposed using a vertical pericardotomy. Ischemia in the left ventricle was induced by ligating the left coronary artery approximately 4 mm from the base of the aorta using a cardiovascular 7.0 monofilament suture on an 11 mm needle (Ethicon Inc.). Fluorescent microspheres (Invitrogen, FluoSpheres 10 μm, 300 μL) were injected into the left ventricular cavity 15 min after the ligation to delineate the ischemic area. The suture was removed 30 min after ligation and the ischemic area was checked to insure reperfusion. The chest was then closed using 5–0 silk sutures for the muscle layers, and wound clips for the cutaneous layer. The animals were allowed to recover from anesthesia under temperature-controlled conditions before they were returned to the colony.

Twenty-four hours after reperfusion, anesthesia was induced using ketamine hydrochloride and the chest was opened. The animals were sacrificed by injecting a 15% potassium chloride solution (w/v) into the left ventricular cavity to arrest the heart in diastole. The heart was excised distal to the aortic valve and washed with saline to remove residual blood. Sagittal slices of the heart were prepared between the base of the ventricle and the apex. Five slices of heart tissue were obtained, each 2 mm thick. The slices were stained by immersion in a 1% solution of 2,3,5-triphenyl-2*H*-tetrazolium chloride (TTC) (Sigma) in isotonic saline. Images of the slices were obtained under bright field microscopy to document tissue TTC staining; and fluorescence microscopic images were generated to observe the deposition of microspheres. The area at risk was delineated by the absence of microspheres and the infarct area was determined by the absence of TTC staining. The AR and MI areas were quantitated to obtain the MI/AR ratio. Statistical analysis (Student's t-test) was determined (data not shown) and the reduction of MI/AR ratio compared to placebo reported.

Acknowledgment

We acknowledge Cedarburg Hauser Pharmaceuticals for their valuable advice and expertise.

References and notes

1. Piper, H. M.; Abdallah, Y.; Schafer, C. *Cardiovasc. Res.* **2004**, *61*, 365.
2. Yellon, D. M.; Hausenloy, D. J. *N. Eng. J. Med.* **2007**, *357*, 1121.
3. Otani, H. *Antioxid. Redox Signaling* **2008**, *10*, 207.
4. Whiteman, M.; Tritschler, H.; Halliwell, B. *FEBS Lett.* **1996**, *379*, 74.
5. Packer, L. *Drug Metab. Rev.* **1998**, *30*, 245.
6. Deng, C.; Sun, Z.; Tong, G.; Yi, W.; Ma, L.; Zhao, B.; Cheng, L.; Zhang, J.; Cao, F.; Yi, D. *PLoS One* **2013**, *8*, e58371.
7. Zweier, J. L. *J. Biol. Chem.* **1988**, *263*, 1353.
8. Werns, S. W.; Lucchesi, B. R. *Cardiovasc. Drugs Ther.* **1989**, *2*, 761.
9. Ganguly, N. K.; Nalini, K.; Wahi, S.; Dhawan, V.; Meenakshi, S.; Chakravarti, R. N. *Mol. Cell. Biochem.* **1992**, *111*, 71.
10. Giordano, F. J. *J. Clin. Investig.* **2005**, *115*, 500.
11. Pugazhenthi, S.; Akhov, L.; Selvaraj, G.; Wang, M.; Alam, J. *Am. J. Physiol. Endocrinol. Metab.* **2007**, *293*, E645.
12. Teichert, J.; Kern, J.; Tritschler, H. J.; Ulrich, H.; Preiss, R. *Int. J. Clin. Pharmacol. Ther.* **1998**, *36*, 625.

13. Schupke, H.; Hempel, R.; Peter, G.; Hermann, R.; Wessel, K.; Engel, J.; Kronbach, T. *Drug Metab. Dispos.* **2001**, *29*, 855.
14. Muller, C.; Dunschede, F.; Koch, E.; Vollmar, A. M.; Kiemer, A. K. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2003**, *285*, G769.
15. Glantzounis, G. K.; Yang, W.; Koti, R. S.; Mikhailidis, D. P.; Seifalian, A. M.; Davidson, B. R. *Curr. Pharm. Des.* **2006**, *12*, 2891.
16. Dulundu, E.; Ozel, Y.; Topaloglu, U.; Sehirli, O.; Ercan, F.; Gedik, N.; Sener, G. *Pharmacology* **2007**, *79*, 163.
17. Duenschede, F.; Erbes, K.; Riegler, N.; Ewald, P.; Kircher, A.; Westermann, S.; Schad, A.; Miesmer, I.; Albrecht-Schock, S.; Gockel, I.; Kiemer, A. K.; Junginger, T. *World J. Gastroenterol.* **2007**, *13*, 3692.
18. Takaoka, M.; Ohkita, M.; Kobayashi, Y.; Yuba, M.; Matsumura, Y. *Clin. Exp. Pharmacol. Physiol.* **2002**, *29*, 189.
19. Sehirli, O.; Sener, E.; Cetinel, S.; Yuksel, M.; Gedik, N.; Sener, G. *Clin. Exp. Pharmacol. Physiol.* **2008**, *35*, 249.
20. Bae, E. H.; Lee, K. S.; Lee, J.; Ma, S. K.; Kim, N. H.; Choi, K. C.; Frokiaer, J.; Nielsen, S.; Kim, S. Y.; Kim, S. Z.; Kim, S. H.; Kim, S. W. *Am. J. Physiol. Renal Physiol.* **2008**, *294*, F272.
21. Koga, H.; Hagiwara, S.; Kusaka, J.; Goto, K.; Uchino, T.; Shingu, C.; Kai, S.; Noguchi, T. *J. Surg. Res.* **2012**, *174*, 352.
22. Freisleben, H. J. *Toxicology* **2000**, *148*, 159.
23. Ko, K. M.; Yiu, H. Y. *Mol. Cell. Biochem.* **2001**, *220*, 141.
24. Oh, S. K.; Yun, K. H.; Yoo, N. J.; Kim, N. H.; Kim, M. S.; Park, B. R.; Jeong, J. W. *Korean Circ. J.* **2009**, *39*, 359.
25. Wang, X.; Yu, Y.; Ji, L.; Liang, X.; Zhang, T.; Hai, C. X. *Food Chem. Toxicol.* **2011**, *49*, 2750.
26. He, L.; Liu, B.; Dai, Z.; Zhang, H. F.; Zhang, Y. S.; Luo, X. J.; Ma, Q. L.; Peng, J. *Eur. J. Pharmacol.* **2012**, *678*, 32.
27. Connell, B. J.; Saleh, M.; Khan, B. V.; Saleh, T. M. *Brain Res.* **2011**, *1375*, 128.
28. Yan, G. T.; Si, Y. I.; Zhang, J. Y.; Deng, Z. H.; Xue, H. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue* **2011**, *23*, 345.
29. Richard, M. J.; Connell, B. J.; Khan, B. V.; Saleh, T. M. *Neurosci. Res.* **2011**, *69*, 299.
30. Bitar, M. S.; Ayed, A. K.; Abdel-Halim, S. M.; Isenovic, E. R.; Al-Mulla, F. *Life Sci.* **2010**, *86*, 844.
31. Ziegler, D.; Nowak, H.; Kempler, P.; Vargha, P.; Low, P. A. *Diabet. Med.* **2004**, *21*, 114.
32. Shashoua, V. E.; Adams, D. S.; Boyer-Boiteau, A.; Cornell-Bell, A.; Li, F.; Fisher, M. *Brain Res.* **2003**, *963*, 214.
33. Guillonneau, C.; Charton, Y.; Ginot, Y. M.; Fouquier-d'Herouel, M. V.; Bertrand, M.; Lockhart, B.; Lestage, P.; Goldstein, S. *Eur. J. Med. Chem.* **2003**, *38*, 1.
34. Rosini, M.; Andrisano, V.; Bartolini, M.; Bolognesi, M. L.; Hrelia, P.; Minarini, A.; Tarozzi, A.; Melchiorre, C. *J. Med. Chem.* **2005**, *48*, 360.
35. Hausenloy, D. J.; Yellon, D. M. *Cardiovasc. Res.* **2004**, *61*, 448.
36. Bolli, R.; Becker, L.; Gross, G.; Mentzer, R., Jr.; Balshaw, D.; Lathrop, D. A. *Circ. Res.* **2004**, *95*, 125.
37. Kobara, M.; Tatsumi, T.; Takeda, M.; Mano, A.; Yamanaka, S.; Shiraishi, J.; Keira, N.; Matoba, S.; Asayama, J.; Nakagawa, M. *Basic Res. Cardiol.* **2003**, *98*, 319.
38. Matsui, T.; Tao, J.; del Monte, F.; Lee, K. H.; Li, L.; Picard, M.; Force, T. L.; Franke, T. F.; Hajjar, R. J.; Rosenzweig, A. *Circulation* **2001**, *104*, 330.
39. Tam, J. P.; Riemen, M. W.; Merrifield, R. B. *Pept. Res.* **1988**, *1*, 6.
40. Packer, L.; Kraemer, K.; Rimbach, G. *Nutrition* **2001**, *17*, 888.
41. Biewenga, G. P.; Haenen, G. R.; Bast, A. *Gen. Pharmacol.* **1997**, *29*, 315.
42. Wolz, P.; Krieglstein, J. *Neuropharmacology* **1996**, *35*, 369.
43. Barltrop, J. A.; Hayes, P. M.; Calvin, M. *J. Am. Chem. Soc.* **1954**, *76*, 4348.
44. Wagner, A. F.; Walton, E.; Boxer, G. E.; Pruss, M. P.; Holly, F. W.; Folkers, K. *J. Am. Chem. Soc.* **1956**, *78*, 5079.
45. Thomas, R. C.; Reed, L. J. *J. Am. Chem. Soc.* **1956**, *78*, 6148.
46. Brown, P. R.; Edwards, J. O. *J. Org. Chem.* **1969**, *34*, 3131.
47. Klatt, M. J.; Niebel, M.; Paust, J.; Rieger, J. US Patent 6,441,024, **2002**.
48. Beisswenger, T.; Bethge, H.; Goede, J.; Hubner, F.; Huthmacher, K.; Klenk, H.; Moller, R. US Patent 5,455,264, **1992**.
49. Hettche, H.; Rischer, M.; Sarlikiotis, W. US Patent 6,348,490, **2002**.
50. Ames, B. N. US Patent 7,030,154 **2006**.
51. Majeed, M.; Nagabhushanam, K. US Patent 7,202,270, 2007.
52. Niebch, G.; Buchele, B.; Blome, J.; Grieb, S.; Brandt, G.; Kampa, P.; Raffel, H. H.; Locher, M.; Borbe, H. O.; Nubert, I.; Fleischhauer, I. *Chirality* **1997**, *9*, 32.
53. Baguisi, A.; Beeuwkes, R.; Casale, R.; Kates, S.A.; Lader, A. manuscripts in preparation.
54. Krucoff, M.W., Ponde, C.K., Hiremath, J., Ajit, M., Ramsaran, E., Patel, M.R., Lader, A.S., Beeuwkes, R., Gibson, C.M., Tcheng, J.E. Presented at the 24th Annual Transcatheter Cardiovascular Therapeutics (TCT) Scientific Symposium, Miami, FL; October 2012, Featured Clinical Research I Session.