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CHEMICAL SYNTHESIS AND ANTI-INFLAMMATORY INVESTIGATIONS OF SOME CYCLIC PEPTIDE DERIVATIVES

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Abstract

The present manuscript describes the design and synthesis of a series of cyclic peptides with their anti-inflammatory activity using mice as the animal model and diclofenac as the reference drug. The research was envisaged to incorporate cyclic and acyclic amino acids through peptide bonds into the basic skeleton for synthesis of final compounds and to explore the effect of these substitutions on inflammation. All the synthesized compounds have been evaluated using paw edema test and they were found to possess significant activity as compared to the normal and standard diclofenac.

Key words: Inflammation, Cyclic Peptides, Diclofenac.

Introduction

Peptides are endogenous substances that play various roles in a living system. Majority of these peptides are linear and serve as important leads in development of novel drugs (Andrews, 2001; Bradbury *et al.*, 2019; Gang *et al*., 2018; Menegatti, 2013; Yamaguchi *et al*., 2013). Chemically, these are composed of two or more amino acid residues joined through peptide bonds, and exhibit diverse biological properties e.g. antifungal, antibacterial, Src Kinase inhibitors, anthelmintic, Na^+/K^+-ATPase inhibitors, antiinflammatory, cytotoxic, antiHIV (Dahiya, 2008; Dellai, 2010; Kimura *et al*., 2012; Gerlach and Mondal, 2012; Sleebs *et al*., 2011). The study of peptides is one of the most active arena of current research due to their diverse medicinal properties (Kaur *et al*., 2012; Mahlapuu *et al*., 2016; Mathison *et al*., 2010; Nguyen *et al*., 2010; Suhas and Gowda, 2012).

The major drawbacks of linear peptides as drugs include metabolic instability, conformational flexibility, non-specificity and hence, poor oral bioavailability and often side effects. These drawbacks can be overcome by a wide range of strategies e.g. cyclization of peptides, peptidomimetics and non-peptide ligands (Choi *et al*., 2020; Goodman *et al*., 1992; Jagtap *et al*., 2016; Li *et al*., 2016; Oh *et al*., 2014). Cyclic peptides containing cyclic ring structure are generally polypeptide chains, that can be synthesized by joining one peptide end with the other through an amide, lactone, ether, thioether and disulfide bond etc.

Cyclic peptides (CPs) have advantages of having lesser degree of freedom within the ring for each constituent, reduced conformational flexibility and stabilized secondary structures. Another benefit is that they acquire the resistance for undergoing hydrolysis using exopeptidases because of the absence of COOH and NH_2 termini. Moreover, these peptides show resistant to endopeptidases, due to less flexibility of structure as compared to linear peptides (Ben Lulu *et al*., 2020; Dougherty *et al*., 2019; Jackson *et al*., 2018; Malde *et al*., 2019; March *et al*., 2019). Out of all cyclic peptides, few can cross the cell membrane. One of classical example of the membrane permeable cyclic peptides is Cyclosporin A.

CPs may have altered pharmacological profile but reduced side effects with respect to their linear analogs. Incorporation of cyclic structures into numerous bioactive peptides lead to highly potent selective analogs (Cai *et al*., 2013; Carredano and Baumann, 2011; Roxin and Zheng, 2012; Touati, 2012). Different cyclization strategies include connection of amino terminus to carboxyl terminus, amino terminus to side chain, side chain

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to carboxyl terminus and side chain to another side chain. Moreover degradation molecules of same are amino acids, that are intoxic (Heinis, 2011; Lambert *et al*., 2011; Papi *et al*., 2016).

CPs are available in minor concentrations in various plants, marine and marine plant sources. Their isolation from the natural sources not only makes them expensive drugs but also can cause a severe ecological imbalance (Luo *et al*., 2018; Yamaguchi *et al*., 2017). They are found to be indispensable part of the drug discovery and drug development. In order to produce such biologically active CPs at commercial scale, synthetic strategies are developed. These include solid phase synthesis and solution phase synthesis. Some examples of natural CPs synthesized through these strategies include cherimolacyclopeptide G, argifin, cyclosquamosin D, dichotomin A analogs, NMe-IB-01212 and tunicyclins C & D (Dixon *et al*., 2009; Roviello *et al*., 2016). The major challenges in synthesizing CPs include synthesis of linear peptide through several protection, coupling and deprotection steps (usually 30 for a pentapeptide) which is subsequently cyclised through esterification. Even a minor error at any stage of this long sequential synthetic process makes the chemist to stop the process and start from the beginning. So far, various research groups have synthesized CPs composed of 5-8 amino acids. Most of them are mainly used to treat cancer, infection, and the diseases associated with metabolic disorders. At present, there are still hundreds of cyclic peptides at the stages of clinical and preclinical studies. It can be predicted that more and more cyclic peptide drugs will constantly be approved into the market in the near future. Till now, several classical reported natural cyclic peptides with antibacterial, anticancer, anti-inflammatory and other biological activities are discussed thoroughly. Therefore the scientist is thought to exploit the anti-inflammatory activity of cyclopeptides by synthesizing it.

Inflammation is a natural defensive response of our body to toxic stimuli, such as injury and infection, that could lead to damage of involved tissues. The inflammatory response includes the activation of different biological mechanisms like the production and secretion of pro-inflammatory mediators such as cytokines, chemokines, interleukins, and growth factors (Villa and Gerwick, 2010). The regulation of the inflammatory process is essential to maintain or restore homeostasis in damaged biological compartments, and alteration of this regulation is associated with different human diseases. On the other hand, several natural peptides with anti- or pro-inflammatory activity have been discovered, in particular, many antimicrobial peptides display anti-inflammatory features, they kill Gram negative and/or positive bacteria, Mycobacterium tuberculosis, fungi, and cancerous cells. In most cases, they are small polycationic peptides able to interact with anionic bacterial surfaces, and able to insert into membrane bilayers, thus forming pores. Alternately, they penetrate into the cell and bind cytoplasmic components crucial to cell living or interfere with cellular metabolism (Gentilucci *et al*., 2010; Roviello *et al*., 2015).

New drug development is aimed at curing or preventing conditions or diseases without suitable therapeutic molecule availability, reducing side effects, improving quality of life, compromising the cost on healthcare systems, while significantly extending patients' lives. Taking into consideration the importance of CPs and their synthetic complications, the objectives of the present study are focused to synthesize selected linear hexapeptides and cyclic peptides and its analogs using protection and deprotection techniques. The synthesized compounds will be evaluated for the anti-inflammatory potential using the most suitable animal pharmacological model.

Materials and Methods

Chemistry

Melting points were determined on a Veego melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Shimadzo (Japan) 8400 S FT-IR spectrophotometer model using nujol and potassium bromide and on a Perkin Elmer RX1 using potassium bromide cell for liquid sample and potassium bromide pellets for solid samples (υ_{max} in cm^{-1}). Proton-NMR spectra were recorded on Brucker multinuclear FT-NMR spectrophotometer model AV-400, 400 MHz using deuterated-chloroform containing tetramethylsilane (Me_4Si) as internal standard (chemical shift in δ, ppm). Synthesis of cyclic peptides was carried out using solution phase peptide technique, protection and deprotection techniques because there are two ends in the amino acids *i.e.* carboxylic and amino terminals. A detailed procedure is given below:

Synthesis of linear hexapeptides

Boc-Gly-Pro-Trp-Tyr-Pro-Phe OMe (KLH)

Compound tripeptide Tyr-Pro-Phe OMe (4.38 gm, 10 mmol) was dissolved in 20 ml of chloroform and 2.3 ml of NMM (21 mmol) was added at 0°C. The reaction mixture was stirred for 15 min. Protected tripeptide Boc-Gly-Pro-Trp OH (4.39 gm, 10 mmol) in 20 ml of chloroform and 2.1 gm of DCC (10 mmol) were added under stirring to the above mixture. After 36 hr, the

reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filtrate. The filtrate was washed with 25 ml of 5% sodium bicarbonate and 25 ml of saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and light petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to give yellow orange semisolid mass of compound **KLH**.

Boc-Gly-Pro-Tyr-Trp-Pro-Phe OMe (KLH1)

Compound tripeptide Trp-Pro-Phe OMe (4.61 gm, 10 mmol) was dissolved in 20 ml of chloroform and 2.3 ml of NMM (21 mmol) was added at 0°C. The reaction mixture was stirred for 15 min. Protected tripeptide Boc-Gly-Pro-Tyr OH (4.16 gm, 10 mmol) in 20 ml of chloroform and 2.1 gm of DCC (10 mmol) were added under stirring to the above mixture. After 36 hr, the reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filtrate. The filtrate was washed with 25 ml of 5% sodium bicarbonate and 25 ml of saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and light petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to give yellow orange semisolid mass of compound KLH1.

Boc-Gly-Pro-Trp-Trp-Pro-Phe OMe (KLH2)

Compound tripeptide Trp-Pro-Phe OMe (4.61 gm, 10 mmol) was dissolved in 20 ml of chloroform and 2.3 ml of NMM (21 mmol) was added at 0°C. The reaction mixture was stirred for 15 min. Protected tripeptide Boc-Gly-Pro-Trp OH (4.39 gm, 10 mmol) in 20 ml of chloroform and 2.1 gm of DCC (10 mmol) were added under stirring to the above mixture. After 36 hr, the reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filtrate. The filtrate was washed with 25 ml of 5% sodium bicarbonate and 25 ml of saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and light petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to give yellow orange semisolid mass of compound KLH2.

Boc-Gly-Tyr-Pro-Tyr-Pro-Phe OMe (KLH3)

Compound tripeptide Tyr-Pro-Phe OMe (4.38 gm, 10 mmol) was dissolved in 20 ml of chloroform and 2.3 ml of NMM (21 mmol) was added at 0°C. The reaction mixture was stirred for 15 min. Protected tripeptide Boc-Gly-Tyr-Pro OH (4.16 gm, 10 mmol) in 20 ml of chloroform and 2.1 gm of DCC (10 mmol) were added under stirring to the above mixture. After 36 hr, the reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filtrate. The filtrate was washed with 25 ml of 5% sodium bicarbonate and 25 ml of saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and light petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to give yellow orange semisolid mass of compound KLH3.

Boc-Gly-Arg-Val-Lys-Ala OMe (KLHA)

Compound dipeptide Gly-Arg-OMe (2.30 gm, 10 mmol) was dissolved in 20 ml of chloroform and 2.3 ml of NMM (21 mmol) was added at 0°C. The reaction mixture was stirred for 15 min. Protected tripeptide Boc-Val-Lys-Ala OH (3.97 gm, 10 mmol) in 20 ml of chloroform and 2.1 gm of DCC (10 mmol) were added under stirring to the above mixture. After 36 hr, the reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filtrate. The filtrate was washed with 25 ml of 5% sodium bicarbonate and 25 ml of saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and light petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to give yellow orange semisolid mass of compound KLHA.

Boc-Gly-Arg-Leu-Lys-Ala OMe (KLHA1)

Compound dipeptide Lys-Ala OMe (2.30 gm, 10 mmol) was dissolved in 20 ml of chloroform and 2.3 ml of NMM (21 mmol) was added at 0°C. The reaction mixture was stirred for 15 min. Protected tripeptide Boc-Gly-Arg-Leu OH (4.25 gm, 10 mmol) in 20 ml of chloroform and 2.1 gm of DCC (10 mmol) were added under stirring to the above mixture. After 36 hr, the reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filtrate. The filtrate was washed with 25 ml of 5% sodium bicarbonate and 25 ml of saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and light petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to give yellow orange semisolid mass of compound KLHA1.

Boc-Gly-Lys-Val-Arg-Ala OMe (KLHA2)

Compound dipeptide Arg-Ala OMe 3t (2.58 gm, 10 mmol) was dissolved in 20 ml of chloroform and 2.3 ml of NMM (21 mmol) was added at 0°C. The reaction mixture was stirred for 15 min. Protected tripeptide Boc-Gly-Lys-Val OH (3.83 gm, 10 mmol) in 20 ml of chloroform and 2.1 gm of DCC (10 mmol) were added under stirring to the above mixture. After 36 hr, the reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filtrate. The filtrate was washed with 25 ml of 5% sodium bicarbonate and 25 ml of saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and light petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to give yellow orange semisolid mass of compound KLHA2.

Boc-Gly-Lys-Leu-Arg-Ala OMe (KLHA3)

Compound dipeptide Arg-Ala OMe (2.58 gm, 10 mmol) was dissolved in 20 ml of chloroform and 2.3 ml of NMM (21 mmol) was added at 0°C. The reaction mixture was stirred for 15 min. Protected tripeptide Boc-Gly-Lys-Leu OH (3.97 gm, 10 mmol) in 20 ml of chloroform and 2.1 gm of DCC (10 mmol) were added under stirring to the above mixture. After 36 hr, the reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filtrate. The filtrate was washed with 25 ml of 5% sodium bicarbonate and 25 ml of saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and light petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to give yellow orange semisolid mass of compound KLHA3.

Cyclo(Gly-Pro-Trp-Tyr-Pro-Phe) KCH

Compound KLH (4.75 gm, 5 mmol) was dissolved in 50 ml of chloroform at 0°C. To The above solution, 0.94 gm of pnp (6.7 mmol) was added and stirred at room temperature for 12 hr. The reaction mixture was filtered and the filtrate was washed with 15 ml of 10% sodium hydrogen carbonate solution until excess of pnp was removed and finally washed with 5% hydrochloric acid (2 ·10 ml) to get the corresponding p-nitrophenyl ester Boc-Gly-Pro-Tyr-Tyr-Pro-Phe-O-pnp.

The resulting intermediate (4.29 gm, 4 mmol) was dissolved in 35 ml of chloroform, 0.91 gm of TFA (8 mmol) was added, stirred at room temperature for 1 hr and washed with 10% sodium bicarbonate solution (2 ·25 ml). The organic layer was dried over anhydrous sodium sulphate to get Gly-Pro-Tyr-Tyr-Pro-Phe-pro-O-pnp, which was dissolved in 25 ml of chloroform and 2.3 ml of NMM (21 mmol) was added. Then all the contents were kept at 0C for 7 days. The reaction mixture was washed with 10% sodium hydrogen carbonate solution until the byproduct p-nitrophenol was removed completely and finally washed with 5% hydrochloric acid (3 ·15 ml). The organic layer was dried over anhydrous sodium sulphate. Finally, chloroform was distilled off and the crude cyclized product was recrystallized from chloroform and n-hexane to give yellow solid of compound.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.16 (t, 6H, pyrrolidine, J_o = 4.00 Hz), 2.28 (m, 8H, pyrrolidine), 3.09 (m, 3H, -CH_2), 3.23 (t, 3H, -CH, J_o = 4.00 Hz), 4.08 (m, 1H, -CH_2), 4.53 (m, 3H, -CH_2), 4.55 (d, 4H, -NH, J_o = 4.84 Hz), 4.92 (m, 1H, -CH_2), 6.86 (m, 4H, ArH), 7.21 (m, 3H, ArH), 7.43 (m, 4H, ArH), 7.52 (m, 2H, ArH) and 8.87 (s, 2H, -OH adjacent to phenyl ring).

(Cyclo(Gly-Pro-Tyr-Trp-Pro-Phe) KCH1

Compound KLH1 (4.86 gm, 5 mmol) was dissolved in 50 ml of chloroform at 0°C. To The above solution, 0.94 gm of pnp (6.7 mmol) was added and stirred at room temperature for 12 hr. The reaction mixture was filtered and the filtrate was washed with 15 ml of 10% sodium hydrogen carbonate solution until excess of pnp was removed and finally washed with 5% hydrochloric acid (2 ·10 ml) to get the corresponding p-nitrophenyl ester Boc-Gly-Pro-Tyr-Trp-Pro-Phe-O-pnp.

The resulting intermediate (4.38 gm, 4 mmol) was dissolved in 35 ml of chloroform, 0.91 gm of TFA (8 mmol) was added, stirred at room temperature for 1 hr and washed with 10% sodium bicarbonate solution (2 ·25 ml). The organic layer was dried over anhydrous sodium sulphate to g*et al*a-Pro-Tyr-Trp-Pro-Phe-O-pnp, which was dissolved in 25 ml of chloroform and 2.3 ml of NMM (21 mmol) was added. Then all the contents were kept at 0°C for 7 days. The reaction mixture was washed with 10% sodium hydrogen carbonate solution until the byproduct p-nitrophenol was removed completely and finally washed with 5% hydrochloric acid (3 ·15 ml). The organic layer was dried over anhydrous sodium sulphate. Finally, chloroform was distilled off and the crude cyclized product was recrystallized from chloroform and n-hexane to give yellow solid of compound (89%), m.p. 198-200 °C.

Spectral and elemental analyses

FT-IRν_{max} (KBr): 3197.98 (N-H stretch), 3055.24 (C-H stretch, aromatic), 2980.02 (C-H stretch, aliphatic), 1655.72 (C=C stretch, aromatic), 1544.98 (C=N stretch),

1492.90 (C-H bend, CH_2), 1355.96 (C-N stretch, aromatic) and 1145.72 (C-N stretch, aliphatic) cm^{-1}.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.16 (t, 6H, pyrrolidine, J_o= 4.00 Hz), 2.28 (m, 8H, pyrrolidine), 3.09 (m, 3H, -CH_2), 3.23 (t, 3H, -C*H*, J_o= 4.84 Hz), 4.08 (m, 1H, -CH_2), 4.53 (m, 3H, -CH_2), 4.55 (d, 4H, -N*H*, J_o= 4.84 Hz), 4.92 (m, 1H, -CH_2), 6.86 (m, 2H, Ar*H*), 7.02 (m, 3H, Ar*H*), 7.21 (m, 3H, Ar*H*), 7.43 (m, 2H, Ar*H*), 7.52 (d, 2H, Ar*H*, J_o= 2.00 Hz), 7.62 (d, 2H, Ar*H*, J_o= 2.00 Hz), 8.87 (s, 1H, -O*H* adjacent to phenyl ring) and 9.43 (s, 1H, -N*H* of pyrrole ring).

(Cyclo(Gly-Pro-Trp-Trp-Pro-Phe) KCH2

Compound KLH2 (4.86 gm, 5 mmol) was dissolved in 50 ml of chloroform at 0°C. To The above solution, 0.94 gm of pnp (6.7 mmol) was added and stirred at room temperature for 12 hr. The reaction mixture was filtered and the filtrate was washed with 15 ml of 10% sodium hydrogen carbonate solution until excess of pnp was removed and finally washed with 5% hydrochloric acid (2 ·10 ml) to get the corresponding p-nitrophenyl ester Boc-Gly-Pro-Trp-Trp-Pro-Phe-O-pnp.

The resulting intermediate (4.38 gm, 4 mmol) was dissolved in 35 ml of chloroform, 0.91 gm of TFA (8 mmol) was added, stirred at room temperature for 1 hr and washed with 10% sodium bicarbonate solution (2 ·25 ml). The organic layer was dried over anhydrous sodium sulphate to get Gly-Pro-Trp-Trp-Pro-Phe-O-pnp, which was dissolved in 25 ml of chloroform and 2.3 ml of NMM (21 mmol) was added. Then all the contents were kept at ^{0}C for 7 days. The reaction mixture was washed with 10% sodium hydrogen carbonate solution until the byproduct p-nitrophenol was removed completely and finally washed with 5% hydrochloric acid (3 ·15 ml). The organic layer was dried over anhydrous sodium sulphate. Finally, chloroform was distilled off and the crude cyclized product was recrystallized from chloroform and n-hexane to give yellow solid of compound (91%), m.p. 181-183°C.

Spectral and elemental analyses

FT-IRν_{max} (KBr): 3150.32 (N-H stretch), 3080.32 (C-H stretch, aromatic), 2985.81 (C-H stretch, aliphatic), 1655.00 (C=C stretch, aromatic), 1535.34 (C=N stretch), 1336.67 (C-N stretch, aromatic) and 1138.00 (C-N stretch, aliphatic) cm^{-1}.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.16 (t, 6H, pyrrolidine, J_o= 4.00 Hz), 2.28 (m, 8H, pyrrolidine), 3.09 (m, 3H, -CH_2), 3.23 (t, 3H, -C*H*, J_o= 4.84 Hz), 4.08 (m, 1H, -CH_2), 4.53 (m, 3H, -CH_2), 4.55 (d, 4H, -N*H*, J_o= 4.84 Hz), 4.92 (m, 1H, -CH_2), 6.86 (m, 4H, Ar*H*), 7.02 (m, 2H, Ar*H*), 7.13 (m, 2H, Ar*H*), 7.21 (m, 1H, Ar*H*), 7.43 (m, 2H, Ar*H*), 7.52 (d, 2H, Ar*H*, J_o= 2.00 Hz), 7.62 (d, 2H, Ar*H*, J_o= 2.00 Hz), 8.87 (s, 1H, -N*H* of pyrrole ring and 9.43 (s, 1H, -N*H* of pyrrole ring).

(Cyclo(Gly-Tyr-Pro-Tyr-Pro-Phe) KCH3

Compound KLH3 (4.36 gm, 5 mmol) was dissolved in 50 ml of chloroform at 0°C. To The above solution, 0.94 gm of pnp (6.7 mmol) was added and stirred at room temperature for 12 hr. The reaction mixture was filtered and the filtrate was washed with 15 ml of 10% sodium hydrogen carbonate solution until excess of pnp was removed and finally washed with 5% hydrochloric acid (2 ·10 ml) to get the corresponding p-nitrophenyl ester Boc- Gly-Tyr-Pro-Tyr-Pro-Phe-O-pnp.

The resulting intermediate (3.98 gm, 4 mmol) was dissolved in 35 ml of chloroform, 0.91 gm of TFA (8 mmol) was added, stirred at room temperature for 1 hr and washed with 10% sodium bicarbonate solution (2 ·25 ml). The organic layer was dried over anhydrous sodium sulphate to get Gly-Tyr-Pro-Tyr-Pro-Phe-O-pnp, which was dissolved in 25 ml of chloroform and 2.3 ml of NMM (21 mmol) was added. Then all the contents were kept at 0°C for 7 days. The reaction mixture was washed with 10% sodium hydrogen carbonate solution until the byproduct p-nitrophenol was removed completely and finally washed with 5% hydrochloric acid (3 ·15 ml). The organic layer was dried over anhydrous sodium sulphate. Finally, chloroform was distilled off and the crude cyclized product was recrystallized from chloroform and n-hexane to give yellow solid of compound (86%), m.p. 152-155°C.

Spectral and elemental analyses

FT-IRν_{max} (KBr): 3177.76 (N-H stretch), 3078.39 (C-H stretch, aromatic), 2993.52 (C-H stretch, aliphatic), 1658.78 (C=C stretch, aromatic), 1529.55 (C=N stretch), 1492.90 (C-H bend, CH_2), 1348.24 (C-N stretch, aromatic) and 1141.96 (C-N stretch, aliphatic) cm^{-1}.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.16 (t, 6H, pyrrolidine, J_o= 4.00 Hz), 2.28 (m, 8H, pyrrolidine), 3.09 (m, 3H, -CH_2), 3.23 (t, 3H, -C*H*, J_o= 4.84 Hz), 4.08 (m, 1H, -CH_2), 4.53 (m, 3H, -CH_2), 4.55 (d, 4H, -N*H*, J_o= 4.84 Hz), 4.92 (m, 1H, -CH_2), 6.86 (m, 4H, Ar*H*), 7.02 (m, 2H, Ar*H*), 7.13 (m, 2H, Ar*H*), 7.21 (m, 1H, Ar*H*), 7.43 (m, 2H, Ar*H*), 7.52 (d, 2H, Ar*H*, J_o= 2.00 Hz) and 8.87 (s, 2H, -O*H* adjacent to phenyl ring).

(Cyclo(Gly-Arg-Val-Lys-Ala) KCHA

Compound KLHA (3.18 gm, 5 mmol) was dissolved in 50 ml of chloroform at 0°C. To The above solution, 0.94 gm of pnp (6.7 mmol) was added and stirred at room temperature for 12 hr. The reaction mixture was filtered and the filtrate was washed with 15 ml of 10% sodium hydrogen carbonate solution until excess of pnp

was removed and finally washed with 5% hydrochloric acid (2 ·10 ml) to get the corresponding p-nitrophenyl ester Boc-Gly-Arg-Val-Lys-Ala-O-pnp.

The resulting intermediate (3.04 gm, 4 mmol) was dissolved in 35 ml of chloroform, 0.91 gm of TFA (8 mmol) was added, stirred at room temperature for 1 hr and washed with 10% sodium bicarbonate solution (2 ·25 ml). The organic layer was dried over anhydrous sodium sulphate to get Gly-Arg-Val-Lys-Ala-O-pnp, which was dissolved in 25 ml of chloroform and 2.3 ml of NMM (21 mmol) was added. Then all the contents were kept at 0°C for 7 days. The reaction mixture was washed with 10% sodium hydrogen carbonate solution until the byproduct p-nitrophenol was removed completely and finally washed with 5% hydrochloric acid (3 ·15 ml). The organic layer was dried over anhydrous sodium sulphate. Finally, chloroform was distilled off and the crude cyclized product was recrystallized from chloroform and n-hexane to give yellow solid of compound.

Spectral and elemental analyses

FT-IRν_{max} (KBr): 3343.32, 3168.62 (N-H stretch, sharp), 2916.37, 2848.56 (C-H stretch, aliphatic), 1683.14 (C=O, amide), 1604.77 (N-H, bend), 1546.91 (C=C stretch, aromatic), 1533.41 (C-N stretch, amide), 1500.62 (C-H bend, CH_2), 1346.31 (C-N stretch, amine) and 991.41 cm^{-1} (C-C, out of plane bend stretch).

^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.96 (m, 6H, -CH_3), 1.46(m, 6H, -CH_2), 1.48(m, 5H, -N*H*), 1.73(m, 4H, -CH_2), 2.13 (s, 1H, -NH_2), 2.52(m, 3H, -CH_3), 3.99(m, 3H, -CH_2), 4.54 (m, 3H, -C*H*), 4.92(m, 5H, -CH_2), 5.33(s, 2H, -NH_2), 8.17 (s, 1H, >N*H*) and 8.87 (s, 2H, -NH_2).

(Cyclo(Gly-Arg-Leu-Lys-Ala) KCHA1

Compound KLHA1 (3.28 gm, 5 mmol) was dissolved in 50 ml of chloroform at 0°C. To The above solution, 0.94 gm of pnp (6.7 mmol) was added and stirred at room temperature for 12 hr. The reaction mixture was filtered and the filtrate was washed with 15 ml of 10% sodium hydrogen carbonate solution until excess of pnp was removed and finally washed with 5% hydrochloric acid (2 ·10 ml) to get the corresponding p-nitrophenyl ester Boc-Gly-Arg-Leu-Lys-Ala-O-pnp.

The resulting intermediate (3.12 gm, 4 mmol) was dissolved in 35 ml of chloroform, 0.91 gm of TFA (8 mmol) was added, stirred at room temperature for 1 hr and washed with 10% sodium bicarbonate solution (2 ·25 ml). The organic layer was dried over anhydrous sodium sulphate to get Gly-Arg-Leu-Lys-Ala-O-pnp (76), which was dissolved in 25 ml of chloroform and 2.3 ml of NMM (21 mmol) was added. Then all the contents were kept at 0°C for 7 days. The reaction mixture was washed with 10% sodium hydrogen carbonate solution until the byproduct p-nitrophenol was removed completely and finally washed with 5% hydrochloric acid (3 ·15 ml). The organic layer was dried over anhydrous sodium sulphate. Finally, chloroform was distilled off and the crude cyclized product was recrystallized from chloroform and n-hexane to give yellow solid of compound (72%), m.p. 161-163°C.

Spectral and elemental analyses

FT-IRν_{max} (KBr): 3290.56, 3207.62 (N-H stretch, sharp), 2931.80, 2848.56 (C-H stretch, aliphatic), 1649.14 (C=O, amide), 1604.77 (N-H, bend), 1546.91 (C=C stretch, aromatic), 1533.41 (C-N stretch, amide), 1500.62 (C-H bend, CH_2), 1346.31 (C-N stretch, amine) and 991.41 cm^{-1} (C-C, out of plane bend stretch).

^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.96 (m, 6H, -CH_3), 1.46 (m, 6H, -CH_2), 1.48 (m, 5H, -N*H*), 1.73 (m, 4H, -CH_2), 2.13 (s, 1H, -NH_2), 2.52 (m, 3H, -CH_3), 3.99 (m, 3H, -CH_2), 4.54 (m, 3H, -C*H*), 4.91 (m, 2H, -CH_2), 4.92 (m, 5H, -CH_2), 5.33 (s, 2H, -NH_2), 8.17 (s, 1H, >N*H*) and 8.77 (s, 2H, -NH_2).

(Cyclo(Gly-Lys-Val-Arg-Ala) KCHA2

Compound KLHA2 (3.18 gm, 5 mmol) was dissolved in 50 ml of chloroform at 0°C. To The above solution, 0.94 gm of pnp (6.7 mmol) was added and stirred at room temperature for 12 hr. The reaction mixture was filtered and the filtrate was washed with 15 ml of 10% sodium hydrogen carbonate solution until excess of pnp was removed and finally washed with 5% hydrochloric acid (2 ·10 ml) to get the corresponding p-nitrophenyl ester Boc-Gly-Lys-Val-Arg-Ala-O-pnp.

The resulting intermediate (3.04 gm, 4 mmol) was dissolved in 35 ml of chloroform, 0.91 gm of TFA (8 mmol) was added, stirred at room temperature for 1 hr and washed with 10% sodium bicarbonate solution (2 ·25 ml). The organic layer was dried over anhydrous sodium sulphate to get Gly-Lys-Val-Arg-Ala-O-pnp, which was dissolved in 25 ml of chloroform and 2.3 ml of NMM (21 mmol) was added. Then all the contents were kept at 0°C for 7 days. The reaction mixture was washed with 10% sodium hydrogen carbonate solution until the byproduct p-nitrophenol was removed completely and finally washed with 5% hydrochloric acid (3 ·15 ml). The organic layer was dried over anhydrous sodium sulphate. Finally, chloroform was distilled off and the crude cyclized product was recrystallized from chloroform and n-hexane to give yellow solid of compound (78%), m.p. 172-175 °C.

Spectral and elemental analyses

FT-IRν_{max} (KBr): 3290.56, 3207.62 (N-H stretch, sharp), 2931.80, 2848.56 (C-H stretch, aliphatic), 1649.14 (C=O, amide), 1604.77 (N-H, bend), 1546.91 (C=C stretch, aromatic), 1533.41 (C-N stretch, amide), 1500.62 (C-H bend, CH_2), 1346.31 (C-N stretch, amine) and 991.41 cm^{-1} (C-C, out of plane bend stretch).

^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.96 (m, 6H, -C*H*$_3$), 1.46 (m, 6H, -C*H*$_2$), 1.48 (m, 5H, -N*H*), 1.73 (m, 4H, -C*H*$_2$), 2.13 (s, 1H, -N*H*$_2$), 2.52 (m, 3H, -C*H*$_3$), 3.99 (m, 3H, -C*H*$_2$), 4.54 (m, 3H, -C*H*), 4.92 (m, 5H, -C*H*$_2$), 5.33 (s, 2H, -N*H*$_2$), 8.17 (s, 1H, >N*H*) and 8.87 (s, 2H, -N*H*$_2$).

(Cyclo(Gly-Lys-Leu-Arg-Ala) KCHA3

Compound KLHA3 (3.28 gm, 5 mmol) was dissolved in 50 ml of chloroform at 0°C. To The above solution, 0.94 gm of pnp (6.7 mmol) was added and stirred at room temperature for 12 hr. The reaction mixture was filtered and the filtrate was washed with 15 ml of 10% sodium hydrogen carbonate solution until excess of pnp was removed and finally washed with 5% hydrochloric acid (2 ·10 ml) to get the corresponding p-nitrophenyl ester Boc-Gly-Lys-Leu-Arg-Ala-O-pnp.

The resulting intermediate (3.12 gm, 4 mmol) was dissolved in 35 ml of chloroform, 0.91 gm of TFA (8 mmol) was added, stirred at room temperature for 1 hr and washed with 10% sodium bicarbonate solution (2 ·25 ml). The organic layer was dried over anhydrous sodium sulphate to get Gly-Lys-Leu-Arg-Ala-O-pnp, which was dissolved in 25 ml of chloroform and 2.3 ml of NMM (21 mmol) was added. Then all the contents were kept at 0°C for 7 days. The reaction mixture was washed with 10% sodium hydrogen carbonate solution until the byproduct p-nitrophenol was removed completely and finally washed with 5% hydrochloric acid (3 ·15 ml). The organic layer was dried over anhydrous sodium sulphate. Finally, chloroform was distilled off and the crude cyclized product was recrystallized from chloroform and n-hexane to give yellow solid of compound (69%), m.p. 146-149°C.

Spectral and elemental analyses

FT-IRν_{max} (KBr): 3166.37 (N-H stretch, sharp), 2916.37, 2848.56 (C-H stretch, aliphatic), 1670.35 (C=O, amide), 1604.77 (N-H, bend), 1573.42 (C=C stretch, aromatic), 1535.34 (C-N stretch, amide), 1500.62 (C-H bend, CH_2), 1346.31 (C-N stretch, amine) and 966.34 cm^{-1} (C-C, out of plane bend stretch).

^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.96 (m, 6H, -C*H*$_3$), 1.46 (m, 6H, -C*H*$_2$), 1.48 (m, 5H, -N*H*), 1.73 (m, 4H, -C*H*$_2$), 2.13 (s, 1H, -N*H*$_2$), 2.52 (m, 3H, -C*H*$_3$), 3.99 (m, 3H, -C*H*$_2$), 4.54 (m, 3H, -C*H*), 4.91 (m, 2H, -C*H*$_2$), 4.92 (m, 5H, -C*H*$_2$), 5.33 (s, 2H, -N*H*$_2$), 8.17 (s, 1H, >N*H*) and 8.77 (s, 2H, -N*H*$_2$).

Pharmacological studies

All the compounds synthesized were evaluated using the disc-diffusion methods and anti-inflammatory behavioral for their anti-bacterial and anti-inflammatory performance respectively.

Anti-inflammatory activity

Animals were divided into different groups. Each group consists of six animals. The anti-inflammatory effect of test compounds was assessed by employing carrageenan induced paw oedema in rats (Winter *et al*, 1962). The different groups of rats were pre-treated with their respective doses. After 1 hour, oedema was induced by administration of 0.1 ml of 1% carrageenan suspension into sub-plantar region of left hind paw of each rat and paw volume was measured by Plethysmometer (Laboratory enterprises, Nasik), at 0, 1, 2, 3, 4 hr. Mean ± SEM for treated and control animals is calculated and compared for each time interval and statistically analyzed.

Different groups are divided for the treatment purposes

Group 1: Normal control; **Group 2:** Inflammation control group received vehicle (0.25% Carboxymethylcellulose); **Group 3:** Standard group treated with Diclofenac sodium (20 mg/kg *p.o.*); **Group 4:** Inflammation + KLH; **Group 5:** Inflammation + KLH1; **Group 6**: Inflammation + KLH2; **Group 7**: Inflammation + KLH3; **Group 8**: Inflammation + KLHA; **Group 9**: Inflammation + KLHA1; **Group 10**: Inflammation + KLHA2; **Group 11**: Inflammation + KLHA3; **Group 12:** Inflammation + KCH; **Group 13:** Inflammation + KCH1; **Group 14**: Inflammation + KCH2; **Group 15**: Inflammation + KCH3; **Group 16**: Inflammation + KCHA; **Group 17**: Inflammation + KCHA1; **Group 18**: Inflammation + KCHA2; **Group 19**: Inflammation + KCHA3.

Results and discussion

It is retrieved from the literature that edema induced by carrageenan is generally used as an experimental model for inflammation and is believed to be biphasic; the first phase is attributed to the release of histamine, serotonin and kinin and the second phase is related to the release of prostaglandins and bradykinins. The experimental animals, rats were divided into four groups (n=6) for assessing the anti-inflammatory activity. Carrageenan was freshly prepared in normal saline (1%) and after 1 h of synthesized compounds administration;

0.1 ml of 1% carrageenan suspension was injected subcutaneously into plantar surface of the left hind paw of experimental animals. The volume of the paw up to ankle joint was measured 1 h prior to the injection (V_0) and hourly up to 3 h (V_t) after the injection of carrageenan, using a plethysmometer.

The percentage inhibition of edema was calculated using these paw volumes, with respect to their controls.

$$\%\ edema\ inhibition = \frac{(V_t\text{-}V_0)\ control - (V_t\text{-}V_0)\ treated}{(V_t - V_0)\ control \times 100}$$

The effect of ethanolic extract of cyclic peptides on carrageenan-induced rat paw edema at different hours of study was compared to that of control and positive control for assessing anti-inflammatory activity on the basis of percent inhibition of paw edema volume. The effect of the cyclic peptides against inflammation produced by these individual mediators was studied and it was found that the inflammation induced due to histamine and dextran was being suppressed. The results of anti-inflammatory activity of the compounds are being expressed in terms of paw volume as shown in the table 1. There are compared with the normal, standard and control for assessing the inflammatory activity of the compounds. It was found that majority of the compounds have shown better results after 3 hours, therefore it was hypothesized that their onset of action is found to be about 3 hours.

Scheme – 1

The one linear compound – KLH3 and its cyclic compound – KCH3 exhibited significant anti-inflammatory activity with respect to control. The compound KLH3 was found to be more significant as compared to control whereas KCH3 was found to be most significant as compared to control. The cyclic compound KCH3 was found to be higher active than linear compound KLH3. The remaining compounds KLH, KLH1, KLH2, KCH, KCH1 and KCH2 did not exhibit activity at any of the tested dose during 4 h of observation as it did not show significant percent inhibition of paw edema in animals with respect to control.

Scheme – 2

The linear compound – KLHA2 and its cyclic compound – KCHA2 exhibited significant anti-inflammatory activity with respect to control. The compounds KLHA2 and KCHA2 were found to be more significant as compared to control. Amongst two bioactive compounds, only one linear compound named KLHA2 was possess highest activity as compared to control and almost equivalent to standard drug during 1, 2, 3 and 4 h

Table 1: Anti-inflammatory data of the synthesized compounds.

TIME	KLH	KLH1	KLH2	KLH3	KCH	KCH1	KCH2	KCH3	Normal	Control (-ve)	Diclofenac
0 h	0.93 ± 0.02	0.95 ± 0.04	0.96 ± 0.01	0.92 ± 0.02	0.93 ± 0.03	0.91 ± 0.02	0.92 ± 0.02	0.90 ± 0.01	0.81 ± 0.01	1.00 ± 0.02	0.88 ± 0.00
1 h	1.14 ± 0.03	1.15 ± 0.02	1.17 ± 0.03	1.13 ± 0.02	1.11 ± 0.03	1.07 ± 0.02	1.06 ± 0.01^{*b}	1.10 ± 0.02	0.81 ± 0.01	1.16 ± 0.03^{***a}	0.98 ± 0.00^{***b}
2 h	1.20 ± 0.03	1.22 ± 0.01	1.20 ± 0.02	1.16 ± 0.04^{*b}	1.21 ± 0.02	1.10 ± 0.02^{***b}	1.18 ± 0.02	1.14 ± 0.02^{**b}	0.81 ± 0.01	1.26 ± 0.02^{***a}	0.93 ± 0.00^{***b}
3h	1.24 ± 0.01	1.21 ± 0.01^{*b}	1.29 ± 0.01	1.16 ± 0.04^{***b}	1.25 ± 0.02	1.20 ± 0.02^{**b}	1.15 ± 0.03^{**b}	1.14 ± 0.02^{**b}	0.82 ± 0.01	1.31 ± 0.02^{***a}	0.84 ± 0.01^{***b}
4 h	1.23 ± 0.01	1.19 ± 0.02^{*b}	1.27 ± 0.03	1.11 ± 0.03^{**b}	1.22 ± 0.03	1.24 ± 0.01	1.10 ± 0.02^{***b}	1.04 ± 0.03^{***b}	0.82 ± 0.01	1.40 ± 0.02^{***a}	0.83 ± 0.01^{***b}
TIME	**KLHA**	**KLHA1**	**KLHA2**	**KLHA3**	**KCHA**	**KCHA1**	**KCHA2**	**KCHA3**	**Normal**	**Control (-ve)**	**Diclofenac**
0 h	0.90 ± 0.02	0.95 ± 0.04	0.99 ± 0.02	0.86 ± 0.02	0.92 ± 0.03	0.88 ± 0.03	0.90 ± 0.03	0.87 ± 0.01	0.81 ± 0.01	1.00 ± 0.02	0.88 ± 0.00
1 h	1.13 ± 0.04	1.16 ± 0.02	0.97 ± 0.03^{***b}	1.18 ± 0.03	1.11 ± 0.03	1.04 ± 0.02^{*b}	1.06 ± 0.01^{*b}	1.18 ± 0.03	0.81 ± 0.01	1.16 ± 0.03^{***a}	0.98 ± 0.00^{***b}
2 h	1.19 ± 0.03	1.24 ± 0.02	0.94 ± 0.01^{***b}	1.26 ± 0.02	1.21 ± 0.02	1.10 ± 0.02^{**b}	1.14 ± 0.03^{*b}	1.25 ± 0.02	0.81 ± 0.01	1.26 ± 0.02^{***a}	0.93 ± 0.00^{***b}
3h	1.25 ± 0.02	1.28 ± 0.01	0.93 ± 0.01^{***b}	1.29 ± 0.01	1.23 ± 0.02	1.14 ± 0.01	1.15 ± 0.03^{**b}	1.18 ± 0.02^{**b}	0.82 ± 0.01	1.31 ± 0.02^{***a}	0.84 ± 0.01^{***b}
4 h	1.24 ± 0.01	1.22 ± 0.05	1.00 ± 0.05^{***b}	1.28 ± 0.01	1.23 ± 0.03	1.16 ± 0.02	0.92 ± 0.01^{***b}	1.13 ± 0.01	0.82 ± 0.01	1.40 ± 0.02^{***a}	0.83 ± 0.01^{***b}

All results are expressed in the form of mean ± SEM (n=5). Signi?cance for % retention was determined by one way ANOVA trailed by Tukey's test. (^{a}) as compared to normal; (^{b}) as compared to control (n = 5). *p>0.05 significant; **p>0.01 more significant; ***p>0.001 most significant.

of observation whereas KCHA2 was found to be significant as compared to control during 1 and 2 h of observation, more significant during 3 h of observation and most significant during 4 h of observation. The remaining compounds KLHA, KLHA1, KLHA3, KCHA, KCHA1 and KCHA3 did not exhibit activity at any of the tested dose during 4 h of observation as it did not show significant percent inhibition of paw edema in animals with respect to control.

Conclusion

Novel derivatives of cyclic peptide have been synthesized following protection and deprotection techniques in which tetrapeptides, pentapeptides, hexapeptides were joined through peptide bonds. Acyclic and cyclic amino acids were chosen depending upon the importance of the same in the biological system. The derivatives were characterized by physicochemical and spectral techniques such as IR, ^{1}H NMR, and Mass. The spectral data obtained was in full agreement with the proposed structures. The *in vivo* evaluation of the newly synthesized compounds has been done through paw edema method. In comparison to the standard drug diclofenac, compounds KCH3 showed a significant decrease in inflammation in the test animals. It was also found that cyclic peptide displayed a good to moderate degree of anti-inflammatory activity as compared to the linear peptide compounds. Thus, these compounds demonstrated better activity in the treatment of inflammatory disorders.

Finally, it can be concluded that in scheme 2, cyclization of KLHA2 showed decline in anti-inflammatory potential of its cyclic derivative KCHA2 with respect to control. But a reverse pattern of activity was observed in scheme 1, cyclization of KLH3 showed increase in anti-inflammatory potential of its cyclic derivative KCH3 with respect to control. Further, it can be concluded that, amongst three experimental schemes, only cyclization in scheme 2 showed better anti-inflammatory activity with respect to control as well as diclofenac.

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References

Andrews, J.M. (2001). Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother.*, **48:** 5-16.

Ben Lulu, M., E. Gaster, A. Libman and D. Pappo (2020). Synthesis of Biaryl Bridged Cyclic Peptides via Catalytic Oxidative Cross Coupling Reactions. *Angew. Chem. Int. Ed.* **59:** 4835-9.

Bradbury, M.S., B. Yoo, U. Wiesner, P. Chen, K. Ma, S.G. Patel and D.K. Zanoni (2019). *U.S. Patent No. 10,335,501*. Washington, DC: U.S. Patent and Trademark Office.

Cai, M., M. Stankova, D. Muthu, A. Mayorov, Z. Yang, D. Trivedi and V.J. Hruby (2013). An unusual conformation of ã-melanocyte-stimulating hormone analogues leads to a selective human melanocortin 1 receptor antagonist for targeting melanoma cells. *Biochem.*, **52:** 752-64.

Carredano, E., & Baumann, H. (2011). Affinity ligands from chemical combinatorial libraries. *Protein Purification: Principles, High Resolution Methods, and Applications*, 259-267.

Choi, J.S. and S.H. Joo (2020). Recent trends in cyclic peptides as therapeutic agents and biochemical tools. *Biomol. Ther.*, **28:** 18-25.

Dahiya, R. (2008). Synthesis, spectroscopic and biological investigation of cyclic octapeptide: cherimolacyclopeptide G. *Turk. J. Chem.*, **32:** 205-15.

Dellai, A., I. Maricic, V. Kumar, S. Arutyunyan, A. Bouraoui and A. Nefzi (2010). Parallel synthesis and anti-inflammatory activity of cyclic peptides cyclosquamosin D and met-cherimolacyclopeptide B and their analogs. *Bioorg. Med. Chem. Lett.*, **20:** 5653-7.

Dixon, M.J., A. Nathubhai, O.A. Andersen, D.M.F. Van-Aalten and I. Eggleston (2009). Solid phase synthesis of cyclic peptide chitinase inhibitors: SAR of the Argifin Scaffold. *Org. Biomol. Chem.*, **7:** 259-68.

Dougherty, P.G., A. Sahni and D. Pei (2019). Understanding cell penetration of cyclic peptides. *Chem. Rev.*, **119:** 10241-87.

Gang D., D.W. Kim and H.S. Park (2018). Cyclic peptides: Promising scaffolds for biopharmaceuticals. *Genes*, **9:** 557-61.

Gentilucci, L., R. De-Marco and L. Cerisoli (2010). Chemical modifications designed to improve peptide stability: incorporation of non-natural amino acids, pseudo-peptide bonds, and cyclization. *Curr. Pharm. Des.*,**16:** 3185-203.

Gerlach, S.L. and D. Mondal (2012). Naturally occurring cyclic peptides and their potential application in HIV therapeutics. *J. Bio. Act. Prod. Nat.*, **2:** 1-29.

Goodman, M., S. Ro, T. Yamazaki, J.R. Spencer, A. Toy, Z. Huang, Y. He and T. Reisine (1992). Topochemical design of bioactive peptides and peptidomimetics. *Bioorg. Khim.*, **18:** 1375-93.

Heinis, C. (2011). Bicyclic peptide antagonists derived from genetically encoded combinatorial libraries. *Chimia*, **65:** 677-9.

Jackson, M.A., E.K. Gilding, T. Shafee, K.S. Harris, Q. Kaas, S. Poon, K. Yap, H. Jia, R. Guarino, L.Y. Chan and T. Durek (2018). Molecular basis for the moleculeion of cyclic

peptides by plant asparaginyl endopeptidases. *Nat. Comm.,* **9:** 2411-5.

Jagtap, P.K.A., D. Garg, T.G. Kapp, C.L. Will, O. Demmer, R. Luhrmann and M. Sattler (2016). Rational design of cyclic peptide inhibitors of U2AF Homology Motif (UHM) domains to modulate Pre-mRNA splicing. *J. Med. Chem.,* **59:** 10190-7.

Kaur, H., A.M. Heapy and M.A. Brimble (2012). Synthesis of the antifungal cyclic peptides tunicyclins C and D. *Synlett*., 2012: 275-7.

Kimura, M., T. Wakimoto, Y. Egami, K.C. Tan, Y. Ise and I. Abe (2012). Calyxamides A and B, cytotoxic cyclic peptides from the marine sponge *Discodermia calyx*. *J. Nat. Prod.,* **75:** 290-4.

Lambert, J.N., J.P. Mitchell and K.D. Roberts (2001). The synthesis of cyclic peptides. *J. Chem. Soc. Perkin Trans.,* **1:** 471-84.

Li, G., S. Kusari, C. Golz, C. Strohmann and M. Spiteller (2016). Three cyclic pentapeptides and a cyclic lipopeptide produced by endophytic *Fusarium decemcellulare* LG53. *Rsc. Adv.,* **6:** 54092-8.

Luo, H., H. Yin, C. Tang, P. Wang and F. Liang (2018). Synthesis of cyclic peptide reniochalistatin E and conformational isomers. *Chin. Chem. Lett.,* **29:** 1143-6.

Mahlapuu, M., J. Hakansson, L. Ringstad and C. Bjorn (2016). Antimicrobial peptides: an emerging category of therapeutic agents. *Front. Cell. Infect. Microbiol.,* **6:** 194-9.

Malde, A.K., T.A. Hill, A. Iyer and D.P. Fairlie (2019). Crystal structures of protein-bound cyclic peptides. *Chem. Rev.,* **119:** 9861-9914.

March, J.B. and E.M. Clark (2019). *U.S. Patent No. 10,328,116*. Washington, DC: U.S. Patent and Trademark Office.

Mathison R.D., J.S. Davison, A.D. Befus and D.A. Gingerich (2010). Salivary gland derived peptides as a new class of anti-inflammatory agents: review of preclinical pharmacology of C-terminal peptides of SMR1 protein. *J. Inflamm.,* **7:** 49-52.

Menegatti, S. (2013). Design, selection and development of novel peptide ligands for bioseparations and diagnostics.

Nguyen, L.T., J.K. Chau, N.A. Perry, L. De Boer, S.A. Zaat and H.J. Vogel (2010). Serum stabilities of short tryptophan- and arginine-rich antimicrobial peptide analogs. *PloS One,* **5:** 154-9.

Oh, D., S.A. Darwish, A.N. Shirazi, R.K. Tiwari and K. Parang (2014). Amphiphilic bicyclic peptides as cellular delivery agents. *Chem. Med. Chem.,* **9:** 2449-53.

Papi, S., N. Urbano, E.R. Obenaus and M. Chinol (2016). The Labeling of peptides with positron-emitting radionuclides: The importance of PET in cancer diagnosis. In *Radionuclide Peptide Cancer Therapy* (pp. 105-136). CRC Press.

Roviello, G.N., C. Vicidomini, V. Costanzo and V. Roviello (2016). Nucleic acid binding and other biomedical properties of artificial oligolysines. *Int. J. Nanomed.,* **11:** 5897-9.

Roviello, G.N., D. Musumeci and V. Roviello (2015). Cationic peptides as RNA compaction agents: A study on the polyA compaction activity of a linear alpha, epsilon-oligo-l-lysine. *Int. J. Pharm.,* **485:** 244-8.

Roxin, A. and G. Zheng (2012). Flexible or fixed: A comparative review of linear and cyclic cancer-targeting peptides. *Fut. Med. Chem.,* **4:** 1601-18.

Sleebs, M., D. Scanlon, J. Karas, R. Maharani and A. Hughes (2011). Total synthesis of the antifungal depsipeptide petriellin A. *J.Org.Chem.,* 76: 6686-93.

Suhas, R. and D.C. Gowda (2012). Design and synthesis of tryptophan containing peptides as potential analgesic and anti inflammatory agents. *J. Pept. Sci.,* **18:** 535-40.

Touati, J. (2012). *Phage Selection of Bicyclic Peptide Ligands and Development of a New Peptide Cyclisation Method* (No. THESIS). EPFL.

Villa, F.A. and L. Gerwick (2010). Marine natural product drug discovery: Leads for treatment of inflammation, cancer, infections, and neurological disorders. *Immunopharmacol. Immunotoxicol.,* **32:** 228-37.

Yamaguchi, S., S. Ito, M. Kurogi-Hirayama and S. Ohtsuki (2017). Identification of cyclic peptides for facilitation of transcellular transport of phages across intestinal epithelium in vitro and in vivo. *J. Control. Release,* **262:** 232-8.

Yamaguchi, S., S. Ito, M. Kurogi-Hirayama and S. Ohtsuki (2017). Identification of cyclic peptides for facilitation of transcellular transport of phages across intestinal epithelium in vitro and in vivo. *J. Control. Release,* **262:** 232-8.