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Peptides *N*-Connected to Hydroxycoumarin and cinnamic acid derivatives: Synthesis and Fluorescence Spectroscopic, Antioxidant and Antimicrobial Properties

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ABSTRACT: The tripeptide Tyr-Gly-Ser and a series of conjugations to coumarin, cinnamic and gallic acid were synthesized in salt form and their antioxidant and antimicrobial activities were investigated. The *N*-connecting hydroxycoumarin, cinnamic and gallic acid derivatives to peptide and using of BBr_3 as demethylating agent on peptide was reported. Their activity was investigated based on the conjugated moiety structures. Studying of their activities showed that, conjugated tripeptides 7,8-dihydroxycoumarin-peptide (**17**), caffeic acid-peptide (**22**) and gallic acid-peptide (**28**) were found to be superior to ascorbic acid with respect to their antioxidant activity, and **12**, **14**, **24**, and **25** exhibited the most antimicrobial activity in the series compared to amoxicillin. Additionally, incredible florescence intensity and brightness of **17** in water and DMSO compare to other synthesized compounds, qualified this peptide as a suitable probe in human body.

Keywords: Hydroxycoumarin-peptide, Hydroxycinnamic-peptide, Gallic acid-peptide, Antioxidant activity, Antimicrobial activity, Fluorescence spectroscopy.

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Introduction

Several reports have described the antioxidant activity of peptides extracted from foods such as whey, casein, soy, egg yolk and other sources, and these peptides can be categorized as safe, clean, and economical natural antioxidants.[1] The antioxidative properties of a peptide depends on its amino acid composition, structure, sequence and hydrophobicity.[2] An important feature of bioactive peptides (BAP), defined as peptides with molecular masses of <6000 Da, is that the human intestine easily absorbs these peptides, especially di- and tripeptides.[3] Absorption of small peptides is 70-80% higher than that of free amino acids.[4] Antimicrobial peptides (AMPs) are another important class of peptides with extended activities that represent a large group of endogenous compounds widely distributed in nature and found in animals, fungi, plants, and bacteria. AMPs have become a model for the discovery of novel antimicrobial drugs that can answer the problem of the increasing antibiotic resistance of pathogenic microorganisms.[5]

Due to the high bioactivities of coumarins, some efforts have focused on the development of novel coumarin-based therapeutic agents.[6] The antioxidant activity of coumarins may include different molecular mechanisms and is probably related to their structural analogy with flavonoids. The position and type of substituent attached to the aromatic ring on the structure of coumarin have a great influence on the antioxidant activity of these compounds. The number of hydroxyl groups on the ring and their positions strongly affect the antioxidant activity of coumarins.[7] Coumarins have low antibacterial activity, but compounds having long-chain hydrocarbons such as ostruthine **1** and ammoresinol **2** with coumarin structures show activity against a wide spectrum of Gram-positive bacteria (Figure 1).[8] Coumarins are also widely used as additives in optical brighteners. They can disperse fluorescent and laser dyes and are used as fluorophore labels on peptides and proteins due to their high-emission quantum yields, photostability, and extended spectral range.[9] Due to the importance of peptides and coumarins separately, the conjunction of these two moieties is an interesting approach in medicinal chemistry. In this regard, some approaches have focused on the development of conjugated peptide-coumarins, Moroder reported the connection of coumarin to the *N*-terminus of a dipeptide containing thiol in a solution for labeling peptides.[10]

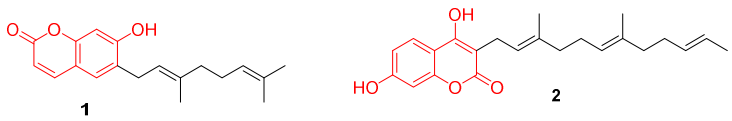

Figure 1. The structures of antibacterial compounds

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In another approach, using cinnamic acid as an open analog of coumarin carboxylic acid, researchers used it as a linker to connect the *C*- and *N*- termini of a peptide to form a cyclic peptide for the metabolic oxidation of CYP3A4.[11] Some methods for solid-phase peptide synthesis of *C*-terminal-modified peptides that also include the coumarin skeleton have been reported.[12] Cu-catalyzed macrocyclization was performed to obtain a macrocyclic coumarin-containing tripeptide for use in thrombin activity measurements.[13] Both hydroxyl and *ortho*-methoxy groups at the coumarin ring connected to the peptide enhanced the activity of coumarin-3-acylamino derivatives in protecting DNA against OH and AAPH-induced oxidation.[14] Recently Freitas *et al* used a triazole as a linker for the synthesis of conjugated coumarin-peptide via copper(I)-catalyzed azide-alkyne cycloaddition. The reported approach has some merits such as low diversity for the starting material; only 7-diethylaminocoumarin was used as sole starting material. Meanwhile, antimicrobial activity of the synthesized conjugated peptide is related to triazole linker, and coupling of coumarin to peptide was done in solution phase and the selected peptide was an amidated *C*-terminal octapeptide.[15]

Additionally, a tripeptide Tyr-Gly-Ser, isolated from peanut showed high antioxidant activity with strong oxygen radical absorbance capacity (ORAC). [16] It displayed a stronger protective effect on linoleic acid peroxidation and H_2O_2-induced oxidative injury in rat pheochromocytoma line PC12 cells than GSH. However, it showed no radical scavenging (DPPH) and no metal cleating (FRAP) activities. In continuation of our research for the synthesis of bioactive peptides,[17] herein, we report an efficient approach for the synthesis of conjugated hydroxycoumarin-, gallic- and -cinnamic acid-tripeptides (Tyr-Gly-Ser) to improve antioxidant activity through DPPH and FRAP methods and their antimicrobial activities and fluorescence spectra were compared.

Results and discussion

Chemistry

To attain the goal for the synthesis of conjugated coumarin-peptides, different coumarin carboxylic acid derivatives **3a-k** were prepared by the reaction of different *o*-hydroxy carbaldehydes and Meldrum's acid refluxing in water[18] (purified yields 82-99%). Compound **4** was synthesized through the nucleophilic addition of salicylaldehyde and maleic anhydride, followed by a cyclization reaction.[19] Compound **8** was selected as a candidate to investigate the effect of the phenolic group. However, for carboxylic acid derivatives containing a hydroxyl moiety in their structure, different additional protection approaches were required

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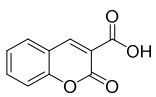
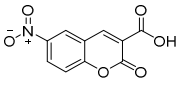
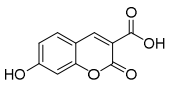
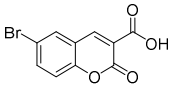
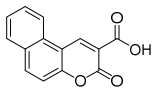
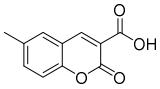
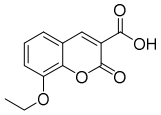
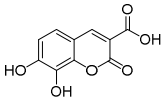
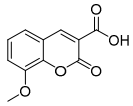
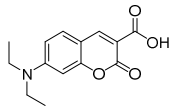
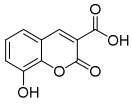
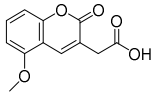
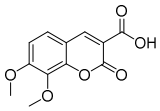
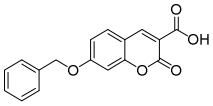
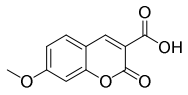
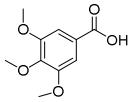
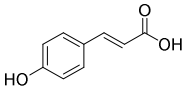
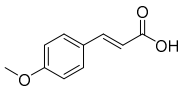
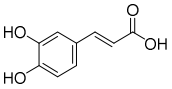
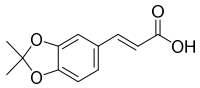
because it is essential to couple them to the tripeptide sequence. For compounds **5**, **6**, **7**, **9a** and **10a**, protection of the free hydroxyl group was done (Table 1). Different known methods were examined for protection of the hydroxyl group in carboxylic acid derivatives, and based on our results, we found that using a methyl ether protected form was the most effective approach.

Table 1. Structures of the starting compounds

 **3a**, 93%	 **3b**, 94%	 **3c**, 91%	 **3d**, 97%	 **3e**, 90%
 **3f**, 98%	 **3g**, 95%	 **3h**, 95%	 **3i**, 99%	 **3j**, 97%
 **3k**. 91%	 **4**, 71%	 **5**, 87%	 **6**, 82%	 **7**, 90%
 8	 9	 **9a**, ,89%	 10	 **10a**, 88%

The linear protected peptide (Fmoc-Tyr (*t*Bu)-Gly-Ser (*t*Bu)) was synthesized on the surface of the 2-chloro-tritylchloride (2-CTC-resin) through a standard solid phase peptide synthesis (SPPS) strategy. After the completion of the peptide sequence, coumarin carboxylic acid **3a** was coupled to the resin-protected peptide. Finally, cleavage from the surface of the resin and final deprotection was performed, yielding desired peptide **11** (Scheme 1). The structure of the synthesized conjugated coumarin-tripeptide **11** was confirmed by NMR spectroscopy, LC-Mass and HRMS spectrometry.

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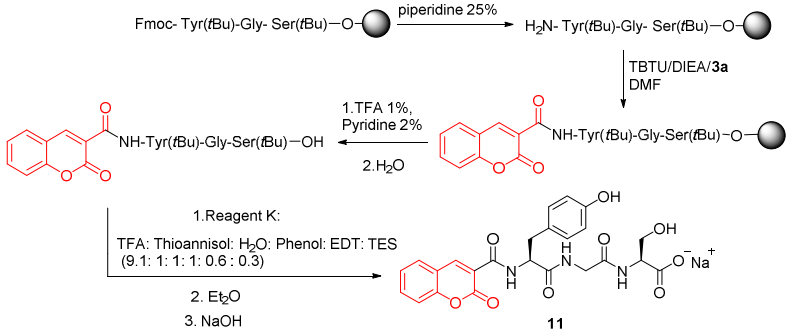
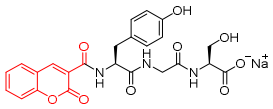
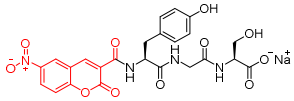
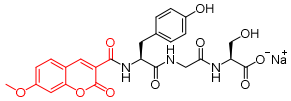
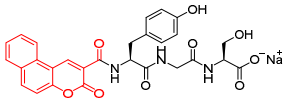
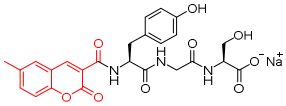
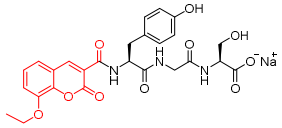
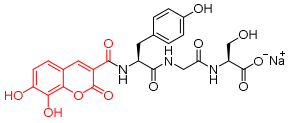
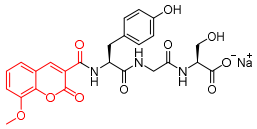
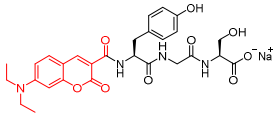
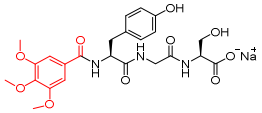
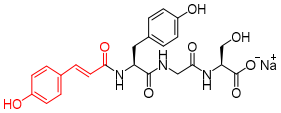
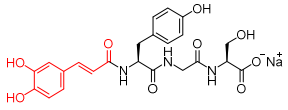
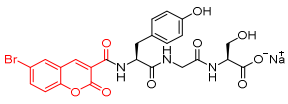
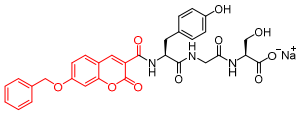
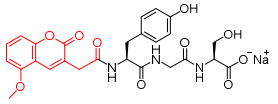


Scheme 1. Synthesis of conjugated coumarin peptide

The data to distinguish the compounds by ^{13}C NMR are related to 3-amidic carbonyls that resonate at 160.5, 168.1, 170.8 ppm and the carbonyl of ester moiety that resonates at 173.2 ppm. The methylene of the tyrosine resonated at two different chemical shifts as diastereotopic hydrogens with different coupling constants (8.6 Hz and 4.6 Hz) with the adjacent hydrogen .

Table 2. Structures of synthesized products

 **11**, 94%	 **12**, 92%	 **13**, 95%
 **14**, 85%	 **15**, 94%	 **16**, 95%
 **17**, 82%	 **18**, 93%	 **19**, 96%
 **20**, 89%	 **21**, 88%	 **22**, 86%
 **23**, 96%	 **24**, 95%	 **25**, 93%

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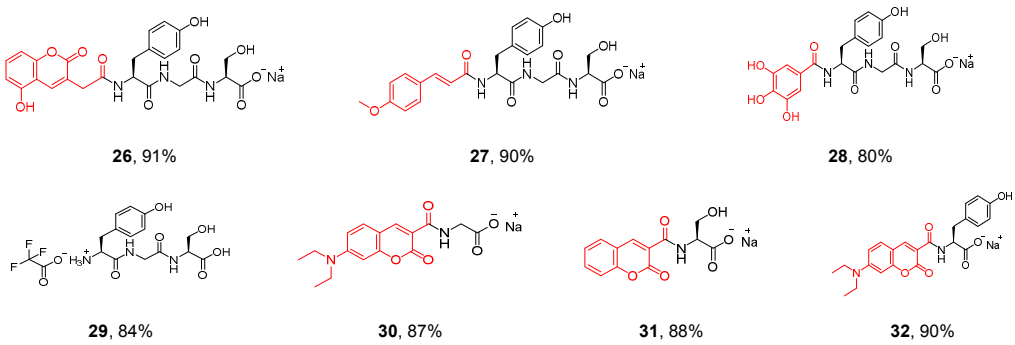

26, 91% **27**, 90% **28**, 80%

29, 84% **30**, 87% **31**, 88% **32**, 90%

Additionally, the HRMS confirmed the synthesis of compound **11**, with a peak at [M+H] 498.1517. The same strategy was used for the synthesis of all conjugated tripeptides **11**-**28**. In the case of peptides **17**, **21**, **26** and **28**, their coumarin starting materials **4**, **5**, **8**, **9a** were protected by a methyl ether group. Because the free hydroxyl has an essential role in the antioxidant activity of the product, different methods were investigated for deprotection of the protecting group, and the best approach was demethylation using BBr_3 in DCM. This step was done before the final deprotection to avoid removing the hydroxyl group of the serine. We tried to do debenzylation of peptide **24** using catalytic hydrogenation reaction conditions, however, in addition to the debenzylation, reduction of the double bond occurred. Using $BF_3.Et_2O$ for debenzylation was not successful either. Conjugation of the coumarin carbocyclic acid to the three amino acids in the tripeptide sequence separately (Ser, Gly, and Tyr) lead to compounds **30**-**32**, and we compared their activity with the synthesized conjugated coumarin-tripeptide. In this way, our aim was to investigate the activity of the products and to determine the role of the individual amino acids in this activity. Table 2 summarizes the structures of synthesized products **11**-**32** (purified yields 80-96%).

Biological test

The *in vitro* antioxidant activity of the starting carboxylic acid derivatives **3a**-**k** and **4**-**10** and conjugated-tripeptides, amino acids **11**-**32** were screened using (1,1-diphenyl-2-picrilhydrazyl) DPPH and ferrous ion-chelating capacity (FRAP) methods. The results obtained from the two approaches were consistent. The ferric reducing antioxidant power assay is based on the reduction of the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) at low pH. The production of Fe^{2+}-TPTZ generates a blue color with a specific sd at 593 nm. The absorbance was plotted against ferrous ion concentrations. L-ascorbic acid was used as the standard antioxidant. The results are expressed as mM ascorbic acid equivalents per mM of each sample.[20] The starting materials

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were divided into two sets based on their FRAP activity. The first set consisted of compounds with lower FRAP effects than ascorbic acid: compounds **3a**, **3b**, **3f**, **7**, and **9** have no FRAP effect, and naphtalenecoumarin carboxylic acid **3e**, 7-hydroxycoumarin carboxylic acid **3c** and trimethoxybenzoic acid **8** have low FRAP effects. These data indicated the important role of coumarin and the position of the methoxy group. 8-Methoxycoumarin **3i** and 8-ethoxy coumarin carboxylic acid **3g** were similar and more effective than 7-methoxycoumarin carboxylic acid **7**. 8-Hydroxycoumarin carboxylic acids **3k** and **4** exhibited the highest antioxidant activity within the first set.

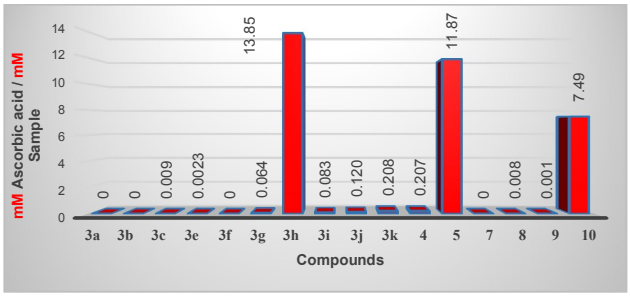

Figure 2. Ferrous ion-chelating capacity activity

In the second set, the compounds that exhibited the highest capacity compared to ascorbic acid was 7,8-dihydroxy coumarin carboxylic acid **3h**, which was 13 times stronger than ascorbic acid and a little better than 7,8-dimethoxy coumarin **5**. The coumarin ring conferred an enhancement of FRAP activity of both compounds compared to dihydroxycinnamic acid **10**. (Figure 2) Tripeptide **11** and **29** had a negligible FRAP effect and peptides **14**, **16**, **18**, **19**, **24**, **25** and **31** had a weak to moderate FRAP effect (Figure 3). Compound **19**, with a dimethylamino group at position 7 on the coumarin ring, had the highest effect among the peptides that had a lesser effect than ascorbic acid. Peptides containing caffeic acid **22**, trihydroxybenzoic acid **28**, and dihydroxycoumarin carboxylic acid **17** exhibited the highest ferrous ion-chelating capacity, 5-6 times more than ascorbic acid, which revealed the contribution of a free phenolic OH group. *p*-Hydroxycinnamic acid **21** is twice as strong as ascorbic acid, but its methoxy analog **27** had a weak effect. The DPPH free-radical inhibition activity assay was performed in accordance with standard protocols.

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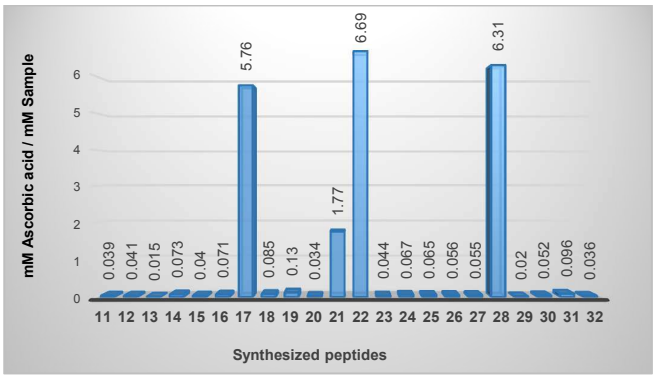

Figure 3. Ferrous ion-chelating capacity activity of peptides

The percent of free-radical inhibition (IC_{50}%) was calculated.[21] Among the compounds in Table 1, only five exhibited considerable radical-scavenging activity (Figure 4). 3-Oxo-3H-benzo[*f*]chromene-2-carboxylic acid **3e** and 8-hydroxycoumarin carboxylic acid **3k** demonstrated moderate to high DPPH capacity. The *ortho*-dihydroxy arrangement in 7,8-dihydroxy carboxylic acid **3h** and caffeic acid **10** and the dimethoxy arrangement in coumarin carboxylic acid **5** enhanced the DPPH activity of these compounds 1.5-3 times compared to ascorbic acid. The great difference in DPPH activity of trimethoxybenzoic acid peptide **20** and its tri-hydroxyl analog **28** reveal the effect of phenolic groups, which was consistent with the high activity of dihydroxy coumarin-tripeptide **17** and cinnamic-tripeptide **22**.

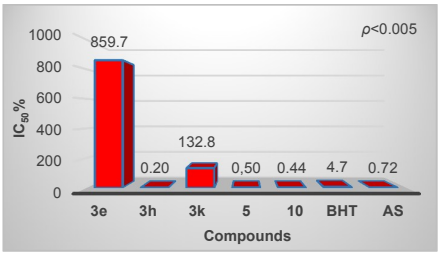

Figure 4. Radical-scavenging activity of the starting compounds

The higher radical-scavenging activity of conjugated-tripeptide **25** compared to BHT and conjugated-tripeptide **18** was conferred by the high stability of the resulting radical. Conjugated coumarin-serine **31** had more powerful antioxidant effect than BHT. The tyrosine analog **32** had the same effect, but due to unexpected interaction in 96-cell plates during the DPPH test, the absorption was not readable (Figure 5).

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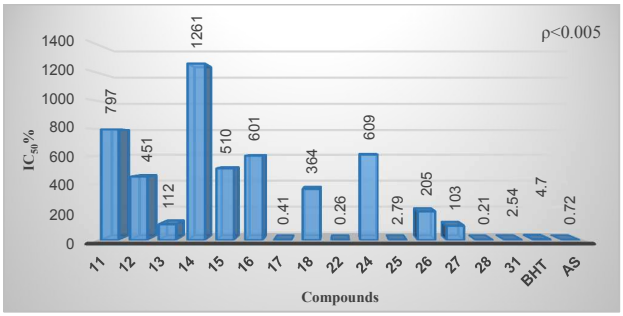

Figure 5. Radical-scavenging activity of synthesized peptides

The *in vitro* antibacterial activity of samples was assessed against *E. coli* ATCC 25922 and *S. aureus* ATCC25923 strains as Gram-negative and Gram-positive bacteria, respectively. Minimum inhibitory concentration (MIC) values were determined using the standard broth micro-dilution technique recommended by the Clinical and Laboratory Standard Institute.[22] The results are shown in Table 3. The MIC results showed that all tested coumarin peptide salts had weak antimicrobial effects against *E. coli* and good to moderate effects against *S. aureus*. Conjugated peptides **12**, **14**, **16**, **24**, **25** and **26** had good antimicrobial effects against the Gram-positive bacterium. Peptides **14** and **24**, with the extra aromatic ring on coumarin backbone, had the highest antibacterial effects. The lowest inhibition of *S. aureus* was related to **13**, **17** and **18**. In the case of the 7-benzyloxy conjugated coumarin-peptide **24**, the effect against *S. aureus* was half as strong as cefixime and higher than amoxicillin. A comparison of the data revealed that the antibacterial effect of peptide **25** is five times more than the conjugated one **18**. Conjugated-tripeptide **25**, containing a methoxy group, demonstrated higher inhibition than **26**, with a hydroxyl group in the same position, and **16**, with ethoxy group, had higher antibacterial activity than **18**, which had a methoxy group at the same position.

Table 3. Antimicrobial activity of synthesized peptides

Sample	MIC [a] (mM)	MIC [b] (mM)	Sample	MIC [a] (mM)	MIC [b] (mM)
11	10	5	**12**	10	0.625
13	10	>5	**14**	10	**0.312**
16	10	1.25	**17**	10	>5
18	10	>5	**19**	10	2.5
22	1	1	**23**	10	1.25
24	10	**0.01**	**25**	10	0.625
26	10	1.25	**27**	20	5
Amoxicilline	0.0082	0.170	Cefixime	0.0088	0.0022

[a] MIC against *E. coli* (ATCC 25922). [b] Minimum inhibition concentration (MIC) against *S. aureus* (ATCC25923)

Because some coumarin derivatives are used as fluorophores in peptides and proteins, we were interested in the fluorescence properties of the novel synthesized compounds in DMSO

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and H_2O. Conjugated coumarin-peptides have absorbance between 215 and 450 nm, and their emission is between 330 and 500 nm, depending on the substituents of the coumarin ring. Absorption ($\lambda_{Abs.}$) with molar extinction coefficients (ε) and fluorescence ($\lambda_{Em.}$) wavelength maxima were measured for the synthesized conjugated peptides (Table 4, supporting information).

Table 4. Fluorescence and absorbance data for the conjugated peptides

No[a]	$\lambda_{Abs.}$[b] (nm) (ε)[c]	$\lambda_{Abs.}$[d] (nm)) (ε)[c]	$\lambda_{Em.}$[b] (nm) (φ)	λ_{Em}[d] (nm) (φ)
11	290 (2.71)	216(11.89),301(3.09)	436 (0.0268)	-
12	275(5.20)	218(12.82),274(5.98)	424(0.0657)	-
13	348(4.07)	218(12.05),350(4.39)	416(0.0275)	408(0.1085)
14	262(1.33),373(0.89)	221(12.51),377(2.89)	446(0.0622)	458(0.1733)
15	298(2.87)	218(11.95), 302(3.01)	438(0.0245)	-
16	313(2.96)	218(12.34),314(3.50)	484(0.0343)	-
17	269(1.91),364(2.49), 446(1.56)	219(12.18),264(2.02), 348(3.47)	498(**0.2125**)	460(**0.1993**)
18	312(3.61)	218(12.21),253(2.51), 314(4.15)	478(0.0346)	-
19	263(2.69),424(**8.36**)	217(12.24),266(2.36), 432(**8.62**)	473(**0.1637**)	485(0.0468)
20	263(1.84)	218(12.27),260(2.36)	426(0.0292)	-
21	281(1.55)	218(11.46),282(1.14), 297(1.04)	434(0.0230)	-
22	289(1.88),325(1.96)	218(12.34),296(2.00), 319(2.13)	418(0.0497)	-
23	288(2.29)	219(11.82),294(2.25), 345(0.93)	434(0.0300)	-
24	348(3.86)	216(11.94),348(3.54)	432(0.0547)	410(0.0894)
25	291(2.35)	219(12.47),252(1.94), 293(2.48)	446(0.0314)	-
26	288(2.69)	217(12.17),253(1.75), 293(2.37)	**422(0.1310)**	-
27	289(2.44)	216(11.94),308(2.58)	434(0.0229)	-
28	273(1.50)	218(12.01), 272(1.48)	424(0.0555)	-
29	263(1.62)	218(11.10),274(0.42)	-	-
31	300(2.10)	217(11.44),299(2.09)	434(0.0372)	413(0.0304)
32	263(2.91),423(7.61)	217(12.15),266(2.13), 429(7.15)	**474(0.1569)**	480(0.0783)

[a] Synthesized conjugated peptide. [b] λ(nm) in DMSO. [c] $cm^{-1}M^{-1}\times10^4$ [d] λ(nm) in H_2O

In all cases more fluorescence intensity observed in DMSO compared to H_2O. Compound **17** had the most fluorescence intensity in both solvents. Conjugated coumarin-peptides showed quantum yields (φ) higher than the original coumarin-3-carboxylic acid derivetives. Emission of compounds in DMSO had an extra peak around 335 nm compare to the same compound in water. Additionally, absorption of compounds in water had an extra peak around 218 nm that is related to amide bonds and their hydrogen bonding with solvent.

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Conclusions

In conclusion, hydroxycoumarins, -cinnamic acids and gallic acid were conjugated to peptides to improve the biological properties and offer interesting opportunity for fluorometric applications. The conjugation of 7, 8-dihydroxy coumarin to the tripeptide **17** had strongly modified its ferrous ion-chelating and radical scavenging activity and also had the best florescence intensity in water and DMSO. The same antioxidant effect was shown in other compounds **22**, **28** containing two and three hydroxyl groups proved that the number and position of phenolic hydroxyl have important effective roles. The synthesized conjugated-peptides had well to moderate antimicrobial activity against *S. aureus* especially for those that are more lipophilic. Compound **24** had better antimicrobial activity compared to amoxicillin. Despite the need for further research, these compounds could be considered as suitable antioxidants for the prevention of oxidative reaction.

Experimental section

General information

^{1}H, ^{13}C NMR spectra were recorded on a Bruker Advance 300 and 400 spectrometers. Chemical shifts are given in ppm from TMS as an internal reference. Flash column chromatography was carried out using silica Gel 60 (particle size 0.04–0.06 mm/230–400 mesh). The abbreviations are given in separate place. The mass spectra were recorded by EI-mass (70 eV), mass (ESI-triple quadrupole), mass (ESI-ion trap), HRMS (ESI-FT-ICR), HRMS (MALDIFT-ICR). Mass spectra are reported as m/z (% of relative intensity). ACN (H_2O < 5 ppm) was purchased from SdS-Carlo Erba and stored under a nitrogen atmosphere. The purification of peptides was done using preparative HPLC (column C18, 7 μm). IR spectra were obtained on an ABB FT-IR FTLA 2000 spectrometer. Fluorescence data were recorded by JASCO spectrofluorometer FP-6500. Absorption of compounds was read with ELISA Microplate Reader Elx-800 BIO-TEK, Bio-Tek Instruments, Inc. Freeze Dryer (Alpha 1-2 LD plus) for drying purified peptide. All starting materials were purchased from Merck and Aldrich and were used without further purification.

General procedure for the synthesis of 3-coumarin carboxylic acid derivatives (3a-k) [18]

In a 25 mL round flask, salicylic aldehyde (1mmol, 152.15 mg) and Meldrum's acid (1.2 mmol, 144 mg) were suspended in 2 mL water, and refluxed for 1h. The precipitated product was filtered and recrystallized in methanol. (Yield 90-99% depending on compound)

Process for the synthesis of 2-(5-methoxy-2-oxo-2H-chromen-3-yl) acetic acid (4)[19]

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A stirred mixture of succinic anhydride (2mmol, 0.2 gr), 3-methoxy salicylic aldehyde (1mmol, 0.152 gr) and triethylamin (3 mmol, 0.021 mL) was heated to 100 °C. Stirring was continued for 30 minutes. The mixture was cooled and washed with HCl 10% (10 mL). The resulted participates were filtered and purified by column chromatography. (Yellow powder, yield 71%)

Process for the methylation of hydroxyl groups of 7, 8-dihydroxy coumarin-3-carboxylic acid 5

In a 25 mL round flask, 7,8-dihydroxy coumarin-3-carboxylic acid (1mmol, 224 mg) was solved in DMF in an ice bath, NaH in mineral oil (3 mmol, 240 mg) was added slowly and the mixture was stirred. Dimethyl sulfate (285 µl, 3 mmol) was added slowly to the resulted mixture and stirred at room temperature for 24h. Then, 5mL HCl (10%) was added to the reaction mixture, and the product was filtered. The filtrated solid was refluxed in NaOH 1M (10 mL) for 1 hour and then cooled and acidify with 5 mL HCl (10%). (Yellow powder, Yield 87%) For **7** and **9a** methylation was performed in the same way .

Synthesis of 7-benzyloxy coumarin 3-carboxilc acid

A mixture of 7-hydroxy coumarin 3-carboxylic acid (10 mmol, 2.06 g) in 30 mL methanol was added to a 50 mL round flask and stirred in an ice bath. Thionyl chloride (30 mmol, 2.19 mL) was added dropwise to the cold mixture to make it completely soluble. The solution was stirred at room temperature overnight, and the solvent was evaporated under vacuum. The remaining solid was recrystallized in methanol. (Blue crystal, yield 90%) The 7-hydroxy-3-coumarin carboxylic acid methyl ester (1 mmol, 0.22 g) was dissolved in *N*, *N*′-dimethyl formamide (2 mL), NaH in mineral oil (1.2 mmol, 48 mg) was added slowly and then benzyl bromide (0.23 mL, 2 mmol) was added. The mixture was stirred overnight. After adding 10 mL of water, the light gray solid was filtered and recrystallized in ethanol (Yield 80%).To methyl 7-benzyloxy coumarin 3-carboxylic acid (1 mmol, 310 mg) in 3 mL methanol, NaOH (3 mmol, 120 mg) in 1 mL H_2O was added and refluxed for 6 hours. The solvent was removed under reduced pressure and the viscous product was acidified with HCl 10% to pH 3. The solid was filtered and recrystallized in ethanol. (Yield 82%, green crystal)

Synthesis of protected caffeic acid 10a[23]

A mixture of caffeic acid methyl ester (2.02 mmol, 392 mg), acetone (9.7 mL), toluene (36 mL), 2, 2-dimethoxy propane (1 mL) and PTSA (19 mg) was refluxed in an apparatus equipped dean-stark trap for 3h. The progress of the reaction was monitored by TLC. The solvent was removed under vacuum, resulting in a green powder. Then, KOH in 10 mL water

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was added to the reaction mixture and was refluxed for 3h. The reaction mixture was cooled using small pieces of ice and the pH of the mixture was adjusted to pH 6 using 2 mL of 10% citric acid.

General process for the synthesis of conjugated peptide coumarin in solid phase

Synthesis was carried out using 2-chlorotrityl chloride resin (2.0 mmol/g) following the standard Fmoc strategy. Fmoc-Ser (t-Bu)-OH (1,92 gr, 3.4 mmol) was attached to the swelled 2-CTC resin (2.000 g) with *N*, *N*-diiopropylethylamine (DIEA, 3.6 mL, 20.8 mmol) in anhydrous DCM-DMF (50 mL, 1:1) at room temperature for 2 h. After filtration, the remaining trityl chloride groups were capped by a solution of DCM/MeOH/DIEA (20:2.5:1.2, 47.5 mL) for 30 min in 3 steps. Then, it was filtered and washed thoroughly with DCM (10 mL), DMF (10 mL) and MeOH (10 mL). The loading capacity was determined by weight after drying the resin under vacuum and was 1.3. The resin-bound Fmoc-amino acid was washed with DMF (10 mL) and treated with 25 % piperidine in DMF (28 mL) for 20 min and the resin was washed with DMF (8 mL) 3 times and DCM (8 mL). Then a solution of Fmoc-Gly-OH (3.4 mmol, 1.0 gr), TBTU (1.1 g, 3.4 mmol), and DIEA (1.58 mL, 9.1 mmol) in 13 mL DMF was added to the resin-bound free amine and shaken for 1 h at room temperature. After completion of coupling, resin was washed with DMF (10 mL) two times. The coupling was repeated as in the same way as for other amino acid of the sequence. In all cases for the presence or absence of free primary amino groups, Kaiser test was used. Fmoc determination was done using UV spectroscopy method. After completion of couplings, resin was washed with DMF (10mL, two times) and DCM (10mL, two times). The produced tripeptide was cleaved from resin by treatment of TFA (1 %) in DCM (200 mL) and neutralization with pyridine (2 %) in MeOH (100 mL) in 5 steps. The solvent was removed under reduced pressure until 2 mL of the solution remained and was added droplet to flask containing 10mL pure water and magnet stirrer. After 30 minutes stirring at room temperature, the precipitated clean protected conjugated peptide was filtered and dried under vacuum in water .

Process for synthesis of 17

In an inert media, to a stirred solution of protected form of conjugated-peptide 17 (0.67 gr, 1mmol) in 3 mL DCM, BBr_3 (1.7 mL, 0.161 mmol) in 1mL DCM was added slowly and for 6h was stirred at room temperature. Then solvent removed under vacuumed and protected peptide was used for next step. The methylation for **21**, **26**, and **28** was done in the same way.

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General process for final deprotection:

Reagent K (TFA 9.1mL: Thioanisol 1mL: Phenol 1mg: H_2O 1mL: EDT 0.6mL: TES 0.3mL) was prepared and was added slowly to the protected conjugated peptide in a 25mL flask in ice bath. After 2 hours of stirring, the solution was evaporated under vacuum. The reminded solution (2mL) was slowly added to a flask containing 20 mL Et_2O and the precipitated peptide was separated by filtration. The dried peptide was converted to its sodium salt by using an equivalent amount of diluted NaOH solution, the further purification was done by preparative HPLC.

Sodium (2-Oxo-2H-chromene-3-carbonyl)-*L*-tyrosylglycyl-*L*-serinate (11) White powder (488 mg, 94%); ^{1}H NMR (300 MHz, DMSO- d_6): δ(ppm) = 2.85 (*dd*, *J* = 14.0, 8.0 Hz, 1H, -CH_2), 3.08 (*dd*, *J* = 14.3, 4.2 Hz, 1H, -CH_2), 3.27 (*dd*, *J*=9.5, 5.4 Hz, 1H, -CH_2), 3.55 (*dd*, *J*= 9.5, 5.4 Hz, 1H, -CH_2), 3.72-3.78 (*m*, 3H, -CH, -CH_2), 4.73 (*td*, *J* = 8.0, 4.5 Hz, 1H, -CH), 5.70 (*s*,1H, OH), 6.62 (*d*, *J* = 8.1 Hz, 2H, H-Ar), 7.02 (*d*, *J* = 8.1 Hz, 2H, H-Ar), 7.43 (*t*, *J* = 7.5 Hz, 1H, H-Ar), 7.50 (*d*, *J* = 8.1 Hz, 2H, H-Ar) 7.78 (*t*, *J* = 7.9 Hz, 1H, H-Ar), 7.97 (*dd*, *J* = 7.8, 1.5 Hz,1H, NH), 8.60 (*t*, *J* = 5.8 Hz, 1H, NH), 8.85 (*s*, 1H, =CH), 8.98 (*d*, J = 7.7 Hz, 1H, NH), 9.32 (*s*, 1H, OH); ^{13}C NMR (75 MHz, DMSO- d_6): δ(ppm) = 37.4, 42.3, 54.7, 58.5, 62.6, 115.1, 116.2, 118.2, 118.4, 125.3, 126.9, 130.3, 130.4, 134.3, 134.4, 148.0, 153.9, 156.1, 160.5, 168.1, 170.8, 173.2; HR-MS (ESI, 0.5eV) m/z= Calculated for $C_{24}H_{24}N_3O_9$ $[M+H]^+$ = 498.1513, Found = 498.1518. Calculated for $C_{24}H_{23}N_3NaO_9$ $[M+Na]^+$ = 520.1332, found = 520.1336.

Sodium (6-Nitro-2-oxo-2H-chromene-3-carbonyl)-*L*-tyrosylglycyl-*L*-serinate (12) Light yellow powder (519 mg. 92%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.86 (*dd*, *J* = 14.0, 7.8 Hz, 1H, -CH_2), 3.04 (*dd*, *J* = 14.0, 4.6 Hz, 1H, -CH_2), 3.62 (*dd*, *J* = 10.9, 4.3 Hz, 1H, -CH_2), 3.70 (*dd*, *J* = 10.9, 4.3 Hz, 1H, -CH_2), 3.82 (*d*, *J* = 5.7 Hz, 2H, -CH_2), 4.29 (*dt*, *J* = 8.5, 4.7 Hz, 1H, -CH), 4.76 (*td*, *J* = 7.7, 4.7 Hz, 1H, -CH), 6.62 (*d*, *J* = 8.3 Hz, 2H, H-Ar), 7.01 (*d*, *J* = 8.2 Hz, 2H, H-Ar), 7.71 (*d*, *J* = 9.2 Hz, 1H, NH), 8.07 (*d*, *J* = 7.8 Hz, 1H, NH), 8.49 (*d*, *J* = 2.9 Hz, 1H, H-Ar), 8.52 (*d*, *J* = 2.5 Hz, 1H, H-Ar), 8.89 (*d*, *J* = 7.7 Hz, 1H, NH), 8.98 (*d*, *J* = 2.8 Hz, -H-Ar), 9.00 (*s*, 1H, =CH), 9.21 (*s*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 37.4, 41.9, 54.6, 54.7, 61.4, 115.1, 117.8, 118.9, 120.3, 126.2, 126.9, 128.4, 130.4, 144.0, 146.9, 156.0, 157.4, 159.6, 160.0, 168.8, 170.7, 171.9; HR-MS (ESI, 0.5eV) *m/z*=

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Calculated for $C_{24}H_{23}N_4O_{11}$ $[M+ H]^+$ = 543.1363, Found = 543.1366. Calculated for $C_{24}H_{22}N_4NaO_{11}$ $[M+Na]^+$ = 565.1183, found = 565.1184.

Sodium (7-Methoxy-2-oxo-2H-chromene-3-carbonyl) tyrosylglycylserinate (13) White powder (522 mg, 95%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.83 (*dd*, *J* = 14.0, 8.3 Hz, 1H, -CH_2), 3.06 (*dd*, *J* = 14.0, 4.6 Hz, 1H, -CH_2), 3.20-3.46 (*m*, 1H, -CH_2, covered by H_2O), 3.56 (*dd*, *J* = 9.5, 5.3 Hz, 1H, -CH_2), 3.67 – 3.80 (*m*, 3H, -CH_2, -CH), 3.89 (*s*, 3H, -OMe), 4.72 (*td*, *J* = 8.0, 4.6 Hz, 1H, -CH), 6.61 (*d*, *J* = 8.3 Hz, 2H, H-Ar), 6.98 – 7.05 (*m*, 3H, H-Ar), 7.10 (*d*, *J* = 2.4 Hz, 1H, H-Ar), 7.48 (*d*, *J* = 6.0 Hz, 1H, H-Ar), 7.87 (*d*, *J* = 8.7 Hz, 1H, NH), 8.55 (*t*, J = 5.9 Hz, 1H, NH), 8.79 (*s*, 1H, -CH), 8.91 (*d*, *J* = 7.8 Hz, 1H, NH), 9.25 (*s*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 35.0, 42.2, 54.4, 54.5, 56.3, 62.6, 100.3, 112.1, 113.8, 114.2, 115.0, 118.9, 120.5, 127.1, 130.3, 155.9, 156.3, 160.9, 160.9, 164.6, 168.0, 170.9, 172.7; HR-MS (ESI, 0.5eV) *m/z* = Calculated for $C_{25}H_{24}N_3O_{10}$ $[M-H]^-$ = 526.1467, Found = 526.1465.

Sodium (3-Oxo-3H-benzo[*f*]chromene-2-carbonyl)-*L*-tyrosylglycyl-*L*-serinate (14) Light yellow powder (484 mg, 84%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.89 (*dd*, *J* = 14.1, 8.5 Hz, 1H, -CH_2), 3.12 (*dd*, *J* = 14.2, 4.5 Hz, 1H, -CH_2), 3.27-3.47 (*m*, 1H, -CH_2, covered by H_2O), 3.59(*dd*, *J* = 10.0, 5.2 Hz, 1H, -CH_2), 3.78 (*t*, *J* = 5.6 Hz, 2H, -CH_2), 3.80 (*q*, *J* = 6.3 Hz, 1H, -CH), 4.79 (*td*, *J* = 7.8, 4.5 Hz, 1H, -CH), 5.48 (*s*, 1H, OH), 6.67 (*d*, *J* = 7.8 Hz, 2H, H-Ar), 7.08 (*d*, *J* = 7.9 Hz, 2H, H-Ar), 7.59 (*d*, *J* = 9.4 Hz, 2H, H-Ar), 7.65 (*t*, *J* = 7.6 Hz, 1H, H-Ar), 7.73 (*t*, *J* = 7.6 Hz, 1H, H-Ar), 8.04 (*d*, *J* = 8.1 Hz, 1H, H-Ar), 8.27(*d*, *J* = 9.0 Hz, 1H, H-Ar), 8.49 (*d*, *J* = 8.5 Hz, 1H, NH), 8.73 (*t*, *J* = 5.9 Hz, 1H, NH), 9.05 (*d*, *J* = 7.4 Hz, 1H, NH), 9.38 (*s*, 1H,=CH), 9.45 (*s*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 34.1, 42.5, 55.0, 55.4, 62.7, 112.6, 115.6, 116.2, 116.3, 116.7, 122.2, 123.7, 125.5, 127.1, 128.9, 129.9, 130.4, 135.9, 143.1, 154.4, 156.2, 160.4, 161.1, 168.3, 171.1, 173.6; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{28}H_{26}N_3O_9$ $[M+H]^+$ = 548.1669, Found = 548.1669. Calculated for $C_{28}H_{25}N_3NaO_9$ $[M+Na]^+$ = 570.1488, found = 570.1486.

Sodium (6-Methyl-2-oxo-2H-chromene-3-carbonyl)-*L*-tyrosylglycyl-*L*-serinate (15) White powder (501mg, 94%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.37 (*s*, 3H, -CH_3), 2.83 (*dd*, *J* = 14.0, 8.4 Hz, 1H, -CH_2), 3.07 (*dd*, *J* = 14.0, 4.6 Hz, 1H, -CH_2), 3.28 (*dd*, *J* = 9.5, 5.4 Hz, 1H, -CH_2), 3.55 (*dd*, *J* = 9.5, 5.4 Hz, 1H, -CH_2), 3.70 – 3.78 (m, 3H, -CH_2, -CH), 4.71 (*td*, *J* = 8.0, 4.5 Hz, 1H, CH), 5.6 (*s*, 1H, OH), 6.59 (*d*, *J* = 8.6 Hz, 2H, H-Ar), 7.0 (*d*, *J* = 8.5

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Hz, 2H, H-Ar), 7.39 (*d*, *J* = 8.5 Hz, 1H, H-Ar), 7.49 (*d*, *J* = 6.0 Hz, 1H, NH), 7.56 (*dd*, *J* = 8.6, 2.1 Hz, 1H, H-Ar), 7.74 (*d*, *J* = 2.1 Hz, 1H, H-Ar), 8.59 (*t*, *J* = 5.9 Hz, 1H, NH), 8.76 (*s*, 1H, =CH), 8.99 (*d*, *J* = 7.7 Hz, 1H, NH), 9.29 (*s*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 27.2, 37.5, 42.1, 53.0, 54.6, 61.9, 115.2, 116.0, 118.0, 118.1, 126.94, 129.9, 130.3, 130.4, 134.7, 148.0, 152.2, 156.1, 160.6, 168.7, 168.8, 170.9, 172.0; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{25}H_{26}N_3O_9$ $[M+H]^+$ = 512.1669, Found = 512.1674, $C_{25}H_{25}N_3NaO_9$ $[M+Na]^+$ = 534.1488, Found = 534.1492.

Sodium (8-Ethoxy-2-oxo-2H-chromene-3-carbonyl)-*L*-tyrosylglycyl-*L*-serinate (16) White powder (545 mg, 95%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 1.41 (*t*, *J* = 6.9 Hz, 3H, -CH_3), 2.85 (*dd*, *J* = 13.9, 8.2 Hz, 1H, -CH_2), 3.07 (*dd*, *J* = 14.0, 4.6 Hz, 1H, -CH_2), 3.30 (*dd*, *J* = 9.6, 5.3 Hz, 1H, -CH_2), 3.56 (*dd*, *J* = 9.6, 5.3 Hz, 1H, -CH_2), 3.67 – 3.82 (m, 3H, -CH_2, -CH), 4.19 (*q*, *J* = 7.0 Hz, 2H,-CH_2), 4.73 (*td*, *J* = 8.0, 4.6 Hz, 1H, -CH), 5.63 (*s*, 1H, OH), 6.62 (*d*, *J* = 8.5 Hz, 2H, H-Ar), 7.02 *d*, *J* = 8.5 Hz, 2H, H-Ar), 7.34 (*AB-q*, *J* = 7.8 Hz, 1H, H-Ar), 7.40 (*dd*, *J* = 6.0, 1.7 Hz, 1), 7.48 (*dd*, *J* = 7.7, 1.5 Hz, 1H, H-Ar), 7.51 (*d*, *J* = 6.1 Hz, 1H, NH), 8.61 (*t*, *J* = 5.8 Hz, 1H, NH), 8.80 (*s*, 1H, =CH), 8.98 (*d*, *J* = 7.7 Hz, 1H, NH), 9.33 (*s*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 14.6, 30.3, 42.8, 54.7, 54.9, 62.0, 64.6, 115.1, 117.2, 118.2, 119.1, 121.3, 125.3, 127.0, 130.4, 143.4, 145.6, 156.1, 159.8, 160.3, 160.6, 168.1, 170.9, 173.1; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{26}H_{26}N_3O_{10}$ $[M-H]^-$ = 540.1624, Found = 540.1620.

Sodium (7, 8-Dihydroxy-2-oxo-2H-chromene-3-carbonyl)-*L*-tyrosylglycyl-*L*-serinate (17) Brown powder (452 mg, 82%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.78 (*dd*, *J* = 14.0, 8.4 Hz, 1H, -CH_2), 2.99 (*dd*, *J* = 14.0, 4.6 Hz, 1H, -CH_2), 3.10-3.70 (*m*, 5H, -CH_2, phenolic OH, alcoholic OH), 3.72 (*d*, *J* = 5.7 Hz, 2H, CH_2), 3.84 (*q*, *J* = 6.2 Hz, 1H, -CH), 4.64 (*td*, *J* = 8.2, 4.5 Hz, 1H, -CH), 6.46 (*d*, *J* = 8.7 Hz, 1H, H-Ar), 6.62 (*d*, *J* = 8.1 Hz, 2H, H-Ar), 7.01 (*d*, *J* = 8.2 Hz, 2H, H-Ar), 7.06 (*d*, *J* = 8.6 Hz, 1H, H-Ar), 7.60 (*d*, *J* = 6.3 Hz, 1H, NH), 8.35 (*s*, 1H, =CH), 8.46 (*t*, *J* = 5.8 Hz, 1H, NH), 8.99 (*d*, *J* = 7.6 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 37.4, 42.3, 54.7, 58.5, 62.6, 115.1, 118.2, 118.4, 125.3, 127.0, 130.0, 132.4, 134.4, 148.0, 153.9, 156.1, 160.5, 168.1, 171.9, 173.2; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{24}H_{22}N_3O_{11}$ $[M-H]^-$ = 528.1260, Found = 528.1258.

Sodium (8-Methoxy-2-oxo-2H-chromene-3-carbonyl)-*L*-tyrosylglycyl-*L*-serinate (18) White powder (511mg, 93%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.86 (*dd*, *J* = 14.0, 8.2 Hz, 1H, -CH_2), 3.07 (*dd*, *J* = 14.0, 4.7 Hz, 1H, -CH_2), 3.40- 3.50 (*m*, 1H, -CH_2, covered

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by H_2O), 3.58 (*dd*, *J* = 9.8, 5.2 Hz, 1H, -CH_2), 3.75 (*dd*, *J* = 5.8, 2.3 Hz, 2H, -CH_2), 3.83 (*q*, *J* = 6.4 Hz, 1H, -CH), 3.90 (s, 3H, -OCH_3), 4.74 (*td*, J = 7.9, 4.6 Hz, 1H, -CH), 6.63 (*d*, *J* = 8.5 Hz, 2H, H-Ar), 7.01 (*d*, *J* = 8.5 Hz, 2H, H-Ar), 7.35 (*dd*, *J*=15.0, 6.07 Hz, 1H, H-Ar), 7.40 (*dd*, *J* = 8.3, 1.5 Hz, 1H, H-Ar), 7.47 (*dd*, *J* = 7.5, 1.6 Hz, 1H, H-Ar), 7.52 (*d*, *J* = 6.4 Hz, 1H, NH), 8.60 (*t*, *J* = 5.8 Hz, 1H, NH), 8.78 (*s*, 1H, =CH), 8.98 (*d*, *J* = 7.7 Hz, 1H, NH), 9.38 (*s*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 37.4, 42.4, 54.6, 54.9, 56.3, 62.6, 101.0, 111.1, 115.1, 119.0, 121.3, 127.0, 130.3, 143.3, 146.3, 148.2, 156.1, 160.2, 160.6, 168.1, 170.8, 173.1, 174.7; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{25}H_{26}N_3O_{10}$ $[M+H]^+$ = 528.1618, Found = 528.16171, Calculated for $C_{25}H_{25}N_3NaO_{10}$ $[M+Na]^+$ = 550.1438, Found = 550.14353.

Sodium (7-(Diethylamino)-2-oxo-2H-chromene-3-carbonyl)-*L*-tyrosylglycyl-*L*-serinate (19) Yellow powder (566mg, 96%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 1.13 (*t*, *J* = 7.0 Hz, 6 H, -CH_3), 2.80 (*dd*, *J* = 14.0, 8.5 Hz, 1H, -CH_2), 3.05 (*dd*, *J* = 14.0, 4.6 Hz, 1H, -CH_2), 3.31 (*dd*, *J* = 9.6, 5.3 Hz, 1H, -CH_2), 3.47 (*q*, *J* = 7.1 Hz, 4H, -CH_2), 3.57 (*dd*, *J* = 9.6, 5.3 Hz, 1H, -CH_2), 3.72 (*d*, *J* = 6.0 Hz, 2H,-CH_2), 3.78 (*dd*, J = 9.8, 3.8 Hz, 1H, -CH), 4.70 (*td*, *J* = 8.1, 4.6 Hz, 1H, -CH), 5.59 (*brs*, 1H, OH), 6.59 (*d*, *J* = 2.3 Hz, 1H. H-Ar), 6.62 (*d*, *J* = 8.6 Hz, 2H, H-Ar), 6.78 (*dd*, *J* = 9.1, 2.4 Hz, 1H, H-Ar), 7.00 (*d*, *J* = 8.6 Hz, 2H, H-Ar), 7.50 (*d*, *J* = 6.1 Hz, 1H, NH), 7.63 (*d*, *J* = 9.1 Hz, 1H, H-Ar), 8.55 (*t*, *J* = 6.0 Hz, 1H, NH), 8.59 (*s*, 1H, =CH), 8.92 (*d*, *J* = 7.7 Hz, 1H, NH), 9.34 (*s*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 12.3, 37.5, 42.3, 44.4, 54.5, 54.6, 62.6, 96.0, 107.60 108.9, 110.2, 115.0, 127.2, 130.2, 131.7, 147.8, 152.6, 155.9, 157.3, 161.6, 161.8, 168.1, 171.2, 172.9; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{28}H_{31}N_4O_9$ $[M-H]^-$ = 567.2097, Found = 567.2094.

Sodium (3, 4, 5-Trimethoxybenzoyl)-*L*-tyrosylglycyl-*L*-serinate (20) White powder (481mg, 89%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.92 (*dd*, *J* = 13.8, 10.8 Hz, 1H, -CH_2), 3.06 (*dd*, *J* = 14.0, 4.1 Hz, 1H, -CH_2), 3.45-3.52 (*m*, 1H, -CH_2, covered by H_2O), 3.59 (*dd*, *J* = 10.0, 5.2 Hz, 1H, -CH_2), 3.68 (*s*, 3H, -OCH_3), 3.75 (*d*, *J* = 4.2 Hz, 2H, -CH_2), 3.80 (*s*, 6H, 2-OCH_3), 3.93 (*q*, *J* = 6.2 Hz, 1H, -CH), 4.62 (*ddd*, *J* = 12.3, 8.0, 4.0 Hz, 1H, -CH), 5.28 (*brs*, 1H, OH), 6.64 (*d*, *J* = 8.3 Hz, 2H, H-Ar), 7.13 (*s*, 2H, H-Ar), 7.16 (*d*, *J* = 8.5 Hz, 2H, H-Ar), 7.60 (*d*, *J* = 6.9 Hz, 1H, NH), 8.61 (*t*, *J* = 5.9 Hz, 1H, NH), 8.85 (*d*, *J* = 8.2 Hz, 1H, NH), 9.43 (*s*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 36.6, 42.6, 55.3, 55.9, 56.0, 60.1, 62.8, 105.2, 115.0, 128.6, 129.4, 130.2, 140.0, 152.5, 155.9, 166.1, 168.3, 172.3, 173.8; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{24}H_{28}N_3O_{10}$ $[M-H]^-$ = 518.1780, Found = 518.1777.

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Sodium ((E)-3-(4-hydroxyphenyl) acryloyl)-*L*-tyrosylglycyl-*L*-serinate (21) White powder (434mg, 88%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.73 (*dd*, *J* = 13.9, 10.2 Hz, 1H, -CH_2), 3.01 (*dd*, *J* = 14.1, 4.1 Hz, 1H, -CH_2), 3.45-3.55 (*m*, 1H, -CH_2, covered by H_2O), 3.58 (*dd*, *J* = 9.8, 5.3 Hz, 1H,-CH_2), 3.68 (*dd*, *J* = 9.8, 5.3 Hz, 1H,-CH_2), 3.73 (*d*, *J* = 5.5 Hz, 2H, -CH_2), 3.87 (*q*, *J* = 6.5 Hz, 1H, -CH), 4.57 (*ddd*, *J* = 10.3, 8.2, 4.2 Hz, 1H, -CH), 5.37 (br*s*, 1H, OH), 6.56 (*d*, *J* = 15.8 Hz, 1H, =CH), 6.64 (*d*, *J* = 8.5 Hz, 2H, H-Ar), 6.95 (*d*, *J* = 8.8 Hz, 2H, H-Ar), 7.07 (*d*, *J* = 8.5 Hz, 2H, H-Ar), 7.30 (*d*, *J* = 15.7 Hz, 1H, =CH), 7.48 (*d*, *J* = 8.8 Hz, 2H, H-Ar), 7.54 (*d*, *J* = 6.6 Hz, 1H, NH), 8.41 (*d*, *J* = 8.3 Hz, 1H, NH), 8.54 (*t*, *J* = 5.8 Hz, 1H, NH), 9.40 (br*s*, 2H, OH);, ^{13}C NMR (75 MHz, DMSO-d_6): 37.0, 42.4, 54.8, 55.1, 62.7, 114.4, 115.3, 119.5, 127.5, 128.1, 129.2, 130.1, 138.8, 155.9, 160.4, 165.4, 168.2, 172.0, 173.4; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{23}H_{24}N_3O_8$ $[M-H]^-$ = 470.1569, Found = 470.1569.

Sodium ((E)-3-(3, 4-Dihydroxyphenyl) acryloyl)-*L*-tyrosylglycyl-*L*-serinate (22) Brown powder (438 mg, 86%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.71 (*dd*, *J* = 14.0, 10.2 Hz, 1H, -CH_2), 3.00 (*dd*, *J* = 14.0, 4.0 Hz, 1H, CH_2), 3.10-3.70 (*m*, 2H, -CH_2 covered by H_2O), 3.72 (*d*, *J* = 5.1 Hz, 2H, -CH_2), 3.85 (*q*, *J* = 6.2 Hz, 1H, -CH), 4.53 (*td*, *J* = 9.1, 8.5, 4.0 Hz, 1H, -CH), 6.39 (*d*, *J* = 15.7 Hz, 1H, =CH), 6.63 (*d*, *J* = 8.1 Hz, 2H, H-Ar), 6.69 – 6.83 (*m*, 2H, H-Ar), 6.93 (*d*, *J* = 1.9 Hz, 1H, H-Ar), 7.07 (*d*, *J* = 8.3 Hz, 2H, H-Ar), 7.15 (*d*, *J* = 15.7 Hz, 1H, =CH), 7.52 (*d*, *J* = 6.7 Hz, 1H, NH), 8.28 (*d*, *J* = 8.2 Hz, 1H, NH), 8.42 (*d*, *J* = 5.9 Hz, 1H, NH), 9.27 (*brs*, 3H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 37.2, 42.5, 54.8, 54.9, 62.6, 114.1, 114.9, 116.0, 118.2, 120.5, 126.3, 128.2, 130.1, 139.6, 145.7, 147.6, 155.8, 165.5, 168.2, 172.1, 173.0; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{23}H_{24}N_3O_9$ $[M-H]^-$ = 486.1518, Found = 486.1515.

Sodium (6-Bromo-2-oxo-2H-chromene-3-carbonyl)-*L*-tyrosylglycyl-*L*-serinate (23) White powder (573 mg, 96%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.85 (*dd*, *J* = 13.9, 8.2 Hz, 1H, -CH_2), 3.07 (*dd*, *J* = 14.0, 4.7 Hz, 1H, CH_2), 3.40-3.51 (*m*, 1H, CH_2, covered by H_2O), 3.56 (*dd*, *J* = 9.6, 5.3 Hz, 1H, -CH_2), 3.73 (*d*, *J* = 5.8 Hz, 2H, -CH_2), 3.75 – 3.84 (*m*, 1H, -CH), 4.73 (*td*, *J* = 7.9, 4.6 Hz, 1H, -CH), 6.62 (*d*, *J* = 8.3 Hz, 2H, H-Ar), 7.01 (*d*, *J* = 8.3 Hz, 2H, H-Ar), 7.46 (*d*, *J* = 9.0 Hz, 1H, H-Ar), 7.50 (*d*, *J* = 6.4 Hz, 1H, NH), 7.87 (*dd*, *J* = 8.9, 2.4 Hz, 1H, H-Ar), 8.23 (*d*, *J* = 2.4 Hz, 1H, H-Ar), 8.58 (*t*, *J* = 5.9 Hz, 1H, NH), 8.79 (*s*, 1H, -CH), 8.93 (*d*, *J* = 7.7 Hz, 1H, NH), 9.30 (*brs*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 37.4, 42.3, 54.6, 54.7, 62.5, 110.6, 115.1, 116.7, 118.5, 119.3, 120.3, 127.0, 130.3, 132.2, 136.4, 153.0, 156.0, 160.0, 160.2, 168.0, 170.7, 172.7; HR-MS (ESI, 0.5eV) *m/z*= Calculated

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for $C_{24}H_{23}BrN_3O_9$ $[M+H]^+$ = 576.0618, Found = 576.0620, Calculated for $C_{24}H_{22}BrN_3NaO_9$ $[M+Na]^+$ = 598.0437, Found = 598.0439.

Sodium (7-(Benzyloxy)-2-oxo-2H-chromene-3-carbonyl)-*L*-tyrosylglycyl-*L*-serinate (24) White powder (594 mg, 95%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.86 (*dd*, *J* = 14.0, 8.2 Hz, 1H, -CH_2), 3.07 (*dd*, *J* = 14.2, 4.8 Hz, 1H, -CH_2), 3.30-3.60 (*m*, 3H, -CH_2O, CH_2N, , covered by H_2O), 3.76 (*qd*, *J* = 16.6, 5.7 Hz, 2H, -CH_2), 4.00 (*dt*, *J* = 7.8, 4.1 Hz, 1H, -CH), 4.77 (*td*, *J* = 7.8, 4.5 Hz, 1H, -CH), 5.24 (*s*, 2H, -CH_2), 6.65 (*d*, *J* = 8.1 Hz, 2H, H-Ar), 7.01 (*d*, *J* = 8.3 Hz, 2H, H-Ar), 7.08 (*dd*, *J* = 8.7, 2.3 Hz, 1H, H-Ar), 7.16 (*d*, *J* = 2.4 Hz, 1H, H-Ar), 7.29 – 7.52 (*m*, 6H, H-Ar), 7.86 (*d*, *J* = 8.8 Hz, 1H, NH), 8.64 (*t*, *J* = 6.0 Hz, 1H, NH), 8.79 (*brs*, 1H, =CH), 8.96 (*d*, *J* = 7.6 Hz, 1H, NH), 9.86 (*brs*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 34.7, 42.4, 54.6, 55.2, 62.6, 70.3, 101.2, 112.2, 114.3, 115.2, 126.9, 128.1, 128.3, 128.6, 130.2, 131.8, 136.0, 148.2, 156.1, 160.9, 161.0, 163.6, 167.6, 170.9, 172.8; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{31}H_{30}N_3O_{10}$ $[M+H]^+$ = 604.1931, Found = 604.1941, Calculated for $C_{31}H_{29}N_3NaO_{10}$ $[M+Na]^+$ = 626.1751, Found = 626.1752.

Sodium (2-(5-Methoxy-2-oxo-2H-chromen-3-yl) acetyl)-*L*-tyrosylglycyl-*L*-serinate (25) White powder (524 mg, 93%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.71 (*dd*, *J* = 14.0, 10.0 Hz, 1H, -CH_2), 2.98 (*dd*, *J* = 14.0, 4.2 Hz, 1H, -CH_2), 3.37 (*d*, *J* = 4.3 Hz, 2H, -CH_2), 3.40-3.60 (*m*, 2H, -CH_2, covered by H_2O), 3.77 (*t*, *J* = 5.3 Hz, 2H, -CH_2), 3.88 (*s*, 3H, -OMe), 3.95 (*q*, *J* = 5.9 Hz, 1H, -CH), 4.48 (*td*, *J* = 8.9, 4.2 Hz, 1H, -CH), 5.24 (*brs*, 1H, OH), 6.65 (*d*, *J* = 8.2 Hz, 2H, H-Ar), 7.06 (*d*, *J* = 8.3 Hz, 2H, H-Ar), 7.12 (*dd*, *J* = 6.5, 2.8 Hz, 1H, H-Ar), 7.18 – 7.31 (*m*, 2H, H-Ar), 7.60 (*d*, *J* = 6.9 Hz, 1H, NH), 7.65 (*s*, 1H, -CH), 8.50 (*t*, *J* = 5.9 Hz, 1H, NH), 8.56 (*d*, *J* = 8.1 Hz, 1H, NH), 9.57 (*brs*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 36.7, 37.8, 43.3, 55.0, 55.7, 56.2, 62.8, 113.6, 115.1, 119.4, 119.7, 123.8, 124.7, 128.1, 130.2, 141.5, 142.1, 146.4, 156.0, 160.7, 168.3, 168.8, 172.0, 173.9; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{26}H_{26}N_3O_{10}$ $[M-H]^-$ = 540.1624, Found = 540.1622.

Sodium (2-(5-Hydroxy-2-oxo-2H-chromen-3-yl) acetyl)-*L*-tyrosylglycyl-*L*-serinate (26) White powder (500mg, 91%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.69 (*dd*, *J* = 14.0, 10.1 Hz, 1H, -CH_2), 2.98 (*dd*, *J* = 14.0, 4.2 Hz, 1H, -CH_2), 3.41-3.52 (*m*, 1H, -CH_2, covered by H_2O), 3.37 (*s*, 2H, -CH_2), 3.57 (*dd*, *J* = 9.7, 5.3 Hz, 1H, -CH_2), 3.72 (*d*, *J* = 5.8 Hz, 2H, -CH_2), 3.80 (*dd*, *J* = 7.2, 5.5 Hz, 1H, -CH), 4.46 (*td*, *J* = 9.3, 4.2 Hz, 1H, -CH), 6.63 (*d*, *J* = 8.5 Hz, 2H, H-Ar), 7.05 (*d*, *J* = 8.3 Hz, 2H, H-Ar), 7.16 (*dd*, *J* = 5.7, 3.5 Hz, 1H, H-Ar), 7.23-7.26(*m*, 2H, H-Ar), 7.50 (*d*, *J* = 6.2 Hz, 1H, NH), 7.68 (*s*, 1H, =CH), 8.40 (*d*, *J* = 6.1 Hz, 1H,

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NH), 8.45 (*d*, *J* = 8.6 Hz, 1H, NH), 9.20 (*brs*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 36.5, 42.4, 54.8, 56.1, 62.6, 113.6, 114.9, 119.3, 119.7, 123.8, 124.5, 128.1, 130.1, 141.4, 142.0, 146.4, 155.8, 157.4, 160.5, 168.1, 168.7, 171.7, 172.9; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{25}H_{24}N_3O_{10}$ $[M-H]^-$ = 526.1467, Found = 526.1468.

Sodium ((E)-3-(4-methoxyphenyl) acryloyl)-*L*-tyrosylglycyl-*L*-serinate (27) White powder (456 mg, 90%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.73 (*dd*, *J* = 14.1, 10.2 Hz, 1H, -CH_2), 3.01 (*dd*, *J* = 14.0, 4.1 Hz, 1H, -CH_2), 3.41-3.49 (*m*, 1H, -CH_2 , covered by H_2O), 3.58 (*dd*, *J* = 9.8, 5.3 Hz, 1H,-CH_2), 3.73 (*d*, *J* = 5.5 Hz, 2H, -CH_2), 3.76 (*s*, 3H, -OMe) 3.87 (*q*, *J* = 6.5 Hz, 1H, -CH), 4.57 (*ddd*, *J* = 10.3, 8.2, 4.2 Hz, 1H, -CH), 5.37 (*brs*, 1H, OH), 6.56 (*d*, *J* = 15.8 Hz, 1H, =CH), 6.64 (*d*, *J* = 8.5 Hz, 2H, H-Ar), 6.95 (*d*, *J* = 8.8 Hz, 2H, H-Ar), 7.07 (*d*, *J* = 8.5 Hz, 2H, H-Ar), 7.30 (*d*, *J* = 15.7 Hz, 1H, =CH), 7.48 (*d*, *J* = 8.8 Hz, 2H, H-Ar), 7.54 (*d*, *J* = 6.6 Hz, 1H, NH), 8.41 (*d*, *J* = 8.3 Hz, 1H, NH), 8.54 (*t*, *J* = 5.8 Hz, 1H, NH), 9.61 (*brs*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 36.9, 42.5, 55.1, 55.3, 62.7, 102.3, 114.4, 114.9, 115.3, 119.5, 127.5, 128.1, 129.2, 129.4, 130.1, 138.8, 155.9, 160.4, 165.4, 168.2, 172.1, 173.4; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{24}H_{26}N_3O_8$ $[M-H]^-$ = 484.1725, Found = 484.1723.

Sodium (3, 4, 5-Trihydroxybenzoyl)-*L*-tyrosylglycyl-*L*-serinate (28) Red powder (399 mg, 80%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.65-4.00 (*m*, 7H, -CH_2, -CH, covered by H_2O), 4.50-4.60 (*m*, 1H, -CH), 6.62 (*d*, *J* = 7.9 Hz, 2H, H-Ar), 6.81 (*s,* 2H, H-Ar), 7.07 (*d*, *J* = 8.0 Hz, 2H, H-Ar), 7.54 (*brs*, 1H, NH), 8.09 (*d*, *J* = 8.7 Hz, 1H,NH), 8.27 (*s*, 1H, =CH), 9.13 (*brs*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 36.5, 42.6, 55.3, 56.0, 60.1, 105.2, 115.0, 128.6, 129.4, 130.2, 140.0, 152.5, 155.9, 166.1, 168.3, 172.3, 173.8; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{21}H_{22}N_3O_{10}$ $[M-H]^-$ = 476.1311, Found = 476.1308.

(S)-1-((2-(((S)-1-carboxy-2-hydroxyethyl) amino)-2-oxoethyl) amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-aminium 2,2,2-trifluoroacetate (29) White powder (369 mg, 84%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.84 (*dd*, *J* = 13.9, 7.4 Hz, 1H, -CH_2), 3.01 (*dd*, *J* = 14.2, 5.3 Hz, 1H, -CH_2), 3.66 (*qd*, *J* = 10.9, 4.8 Hz, 2H, -CH_2), 3.85 (*dd*, *J* = 5.6, 2.9 Hz, 2H, -CH_2), 3.99 (*t*, *J* = 6.6 Hz, 1H, -CH), 4.26 (*dt*, *J* = 8.4, 4.8 Hz, 1H, -CH), 6.20 (*brs*, 4H, -OH, NH_2), 6.70 (*d*, *J* = 8.0 Hz, 2H, H-Ar), 7.06 (*d*, *J* = 8.2 Hz, 2H, H-Ar), 8.14 (*d*, *J* = 7.7 Hz, 1H, NH), 8.74 (*t*, *J* = 5.7 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 36.5, 42.0, 54.0, 55.2, 61.7, 115.6, 125.1, 130.7, 156.8, 158.6, 159.0, 159.1, 168.4, 168.9,

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172.3; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{14}H_{20}N_3O_6$ $[M+H]^+$ = 326.1352, Found = 326.1347.

Sodium (7-(Diethylamino)-2-oxo-2H-chromene-3-carbonyl) glycinate (30) Yellow powder (296 mg, 87%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 1.14 (*t*, *J* = 7.0 Hz, 6H, -$2CH_3$), 3.40 – 3.55 (*m*, 6H, -$2CH_2$N, -CH_2NH), 6.61 (*d*, *J* = 2.4 Hz, 1H, H-Ar), 6.78 (*dd*, *J* = 9.1, 2.5 Hz, 1H, H-Ar), 7.66 (*d*, *J* = 8.9 Hz, 1H, H-Ar), 8.62 (*s*, 1H, =CH), 9.01 (*t*, *J* = 4.4 Hz, 1H, NH);^{13}C NMR (75 MHz, DMSO-d_6): low solubility, HR-MS (ESI, 0.5eV) *m/z* = Calculated for $C_{16}H_{17}N_2O_5$ $[M-H]^-$ = 317.1143, Found = 317.1142.

Sodium (2-Oxo-2H-chromene-3-carbonyl)-*L*-serinate (31) White powder (263 mg, 88%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 3.46 (*dd*, *J* = 9.8, 7.1 Hz, 1H, -CH_2), 3.75 (*dd*, *J* = 9.7, 4.9 Hz, 1H, -CH_2), 3.99 (*dt*, *J* = 6.9, 5.2 Hz, 1H, -CH), 5.44 (*brs*, 1H, OH), 7.42 (*t*, *J* = 7.5 Hz, 1H, H-Ar), 7.49 (*d*, *J* = 8.4 Hz, 1H, H-Ar), 7.73 (*t*, *J* = 7.1 Hz, 1H, H-Ar), 7.97 (*dd*, *J* = 7.8, 1.6 Hz, 1H, H-Ar), 8.89 (*s*, 1H, =CH), 9.32 (*d*, *J* = 5.9 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 55.8, 62.4, 116.1, 118.5, 118.8, 125.1, 130.3, 134.0, 147.6, 154.0, 160.1, 160.2, 172.9; HR-MS (ESI, 0.5eV) *m/z*= Calculated. for $C_{13}H_{10}NO_6$ $[M-H]^-$ = 276.0508, Found = 276.0501.

Sodium (7-(Diethylamino)-2-oxo-2H-chromene-3-carbonyl)-*L*-tyrosinate (32) Yellow powder (381 mg, 90%); ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 1.13 (*t*, *J* = 7.0 Hz, 6H, -$2CH_3$), 2.91 (*dd*, *J* = 13.4, 5.1 Hz, 1H, -CH_2), 3.03 (*dd*, *J* = 13.4, 5.4 Hz, 1H, -CH_2), 3.46 (*q*, *J* = 7.2 Hz, 4H, -CH_2), 4.20 (*q*, *J* = 5.2 Hz, 1H, -CH), 6.53 (*d*, *J* = 8.4 Hz, 2H, H-Ar), 6.58 (*d*, *J* = 2.3 Hz, 1H, H-Ar), 6.77 (*dd*, *J* = 9.0, 2.4 Hz, 1H, H-Ar), 6.89 (*d*, *J* = 8.4 Hz, 2H, H-Ar,), 7.64 (*d*, *J* = 9.0 Hz, 1H, H-Ar), 8.62 (*s*, 1H, =CH), 9.01 (*d*, *J* = 6.8 Hz, 1H, NH), 9.25 (*brs*, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) =12.3, 36.9, 44.3, 56.2, 95.9, 107.6, 109.9, 110.3, 114.5, 129.2, 130.3, 131.4, 133.9, 147.2, 152.2, 155.4, 157.1, 160. 6, 161.1, 173.1; HR-MS (ESI, 0.5eV) *m/z*= Calculated for $C_{23}H_{23}N_2O_6$ $[M-H]^-$ = 423.1556, Found = 423.1559.

Sodium N-(((S)-2-(6-bromo-2-oxo-2H-chromene-3-carboxamido)-3-(4-(tertbutoxy)phenyl) propanoyl)glycyl)-O-(tert-butyl)-*L*-serinate White powder, ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) 0.81 (*s*, 9H, *t*-Bu), 1.08 (*s*, 9H, *t*-Bu), 2.87 (*dd*, *J* = 14.0, 7.8 Hz, 1H,-CH_2), 3.05 (*dd*, *J* = 14.0, 4.8 Hz, 1H, -CH_2), 3.50 (*dd*, *J* = 9.5, 4.0 Hz, 1H, -CH_2), 3.63 (*t*, *J* = 4.9 Hz, 1H, -CH_2), 3.83 (*t*, *J* = 5.5 Hz, 2H, -CH_2), 4.38 (*dt*, *J* = 8.7, 4.3 Hz, 1H, -CH), 4.77 (*q*, J = 7.0 Hz, 1H,-CH), 5.41 (br*s*, 1H, OH), 6.63 (*d*, *J* = 8.0 Hz, 2H, H-Ar), 7.01

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(*d*, *J* = 8.0 Hz, 2H, H-Ar), 7.42 (*d*, *J* = 8.8 Hz, 1H, H-Ar), 7.83 (*dd*, *J* = 9.0, 3.0, 1H, H-Ar), 7.91 (*d*, *J* = 8.0 Hz, 1H, H-Ar), 8.18 (s, 1H, H-Ar), 8.52 (*t*, *J* = 5.8 Hz, 1H, NH), 8.78 (*s*, 1H, =CH), 8.96 (*d*, *J* = 7.7 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) = 27.2, 37.5, 42.0, 53.0, 54.6, 61.8, 72.9, 115.2, 116.9, 118.5, 119.3, 120.3, 126.9, 130.4, 132.3, 136.6, 146.8, 153.0, 156.1, 160.3, 168.8, 170.8, 171.9.

DPPH Assay: [21]

Ninety-six-well plates were used to evaluate five different concentrations of each sample. Each concentration was evaluated in triplicate. A 200 µM solution of DPPH was prepared in methanol and added to different concentrations of samples to final concentrations of 40 µM. Sample blanks were prepared by adding methanol instead of DPPH solution to different concentrations of samples. Plates were incubated at 25 ºC for 45 min (Heidolph titramax 1000 and incubator 1000, Germany), and absorbances were recorded at 517 nm (Powerwave XS Microplate spectrophotometer, Bio-Tek Instruments, Inc.). The percent of free-radical inhibition (In %) was calculated as below :

$$\text{In\%} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100$$

Where Ablank is the absorbance of the control reaction (DPPH solution), and Asample is the mean absorbance of test compounds minus the mean absorbance of each corresponding blank. Subsequently, the concentration of solution that resulted in 50% inhibition (IC_{50}) was calculated by plotting inhibition percentage against each sample concentration. Butylated hydroxytoluene (BHT) was assessed as a standard antioxidant.

FRAP Assay:[20]

The ferric reducing antioxidant power assay is based on the reduction of ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex to ferrous tripyridyltriazine (Fe^{2+}–TPTZ) at low pH. The production of Fe^{2+}–TPTZ generates a blue color at 593 nm absorbance (Chen et al. 2006). FRAP reagent was prepared prior to each experiment by mixing solutions a, b, and c (a: Acetate buffer 300 mM pH 3.6, b: 10 mM TPTZ (2, 4, 6-tripyridyl-s- triazine) in 40 mM HCl, c: 20 mM $FeCl_3$. 6 H_2O) at a 10:1:1 ratio. Then, 20 µL of 20 mM stock solution of each sample was mixed with 200 µl of FRAP reagent. The mixture was maintained at room temperature for 10 min. Absorbance was then recorded at 593 nm (Powerwave XS Microplate spectrophotometer, Bio-Tek Instruments, Inc.). Different concentrations of $FeSO_4.7H_2O$ (200, 400, 800, 1200, and 1600 µM) were used as standard solutions and

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reacted with TPTZ reagent. Absorbance was plotted against ferrous ion concentrations. L-ascorbic acid was used as the standard antioxidant. Results were expressed as mM ascorbic acid equivalents per mM of each sample (Chen et al. 2006).

Antibacterial activity assay:[22]

Briefly, 20 mM sample stock solution in DMSO was used to prepare a serial dilution of 8–0.06 mM in sterile Mueller Hinton broth medium in 96-well plates. An overnight culture of each strain on nutrient agar plate was used to prepare bacterial suspensions equal to 0.5 McFarland standards. The suspensions were further diluted prior to addition to each well. Thus, the final bacterial cell concentration in each well was approximately 0.5-1×106 cfu/mL. Cefixime and Amoxicillin were used as the reference antibiotic, and all experiments were conducted in triplicate.

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References and notes:

1 R. J. Elias, S. S. Kellerby, E. A. Decker, *Food Sci. Nutr.,* 2008, **48**, 430-441.

2 a) R. Nahas, *J. Appl. Food Protein Chem.*, John Wiley & Sons, 2015; b) B. H. Sarmadi, A. Ismail, *Peptides* 2010, **31**, 1949-1956; c) C. F. Chi, F. Y. Hu, B. Wang, Z. R. Li, H. Y. Luo, *Mar. Drugs* 2015, **13**, 2580-2601.

3 a) D, B. A. Silk, P, D. Fairclough, M, L. Clark, JPENs *J. Parenteral Enteral Nutr.,* 1980, **4**, 548-553; b) G, H. Li, G, W. Le, Y, H. Shi, S. Shrestha, *Nutr. Res*. 2004, **24**, 469-486.

4 H. Hara, R. Funabiki, M. Iwata, *J. Nutr*., 1984, **114**, 1122-1129.

5 H. S. Won, J. H. Kim, T. Mishig-Ochair, B. J. Lee, *Molecules* 2012, **17**, 12276-12286.

6 G. Xi, Z. Liu, *J. Agric. Food Chem.,* 2015, **63**, 3516-3523.

7 a) B. M. Bizzarri, L. Botta, E. Capecchi, I. Celestino, P. Checconi, A. T. Palamara, L. Nencioni, R. Saladino, *J. Nat. Prod.,* 2017, **80**, 3247-3254; b) B. G. Bubols, B. G. x Damiana, A. Medina-Remon, *Mini. Rev. Med. Chem*., 2013, **13**, 318-334; c) R. O′ Kennedy, R. D. Thornes, *Coumarins Biology, Applications and Mode of Actions*. Wiley & Sons. New York, 1997.

8 S, M. Souza, F, D. Monache, A. Z. Smânia, *Z. Naturforsch. C*., 2005, **60**, 693-700.

9 a) D. B. Wagner, *Molecules* 2009, **14**, 210-237; b) A, R. Katritzky, M. Yoshioka, T. Narindoshvili, A. Chung, J. V. Johnson, *Org. Biomol. Chem*. 2008, **6**, 4582-4586.

10 L. Moroder, G. Bovermann, G. Mourier, W. Gohring, *Biol. Chem*., 1987, **368**, 831-838.

View Article Online
DOI: 10.1039/C8NJ00383A

11 R. Nofsinger, T. Fuchs-knotts, R. T. Borchardt, *J. Pharm. Sci.,* 2012, **101**, 3486-3499.

12 a) P, D. Edwards, R, C. Mauger, K, M. Cottrell, F, X. Morris, K. K. Pine, M, A. Sylvester, C. W. Scott, S. T. Furlong, *Bioorg. & Med. Chem. Lett*., 2000, **10**, 2291-2294; b) O. García, E. Nicolás, F. Albericio, *Tetrahedron Lett.,* 2003, **44**, 5319-5321.

13 S. Sander, V. Berkel, B. V. Lee, L. Floris, *Chem. Commun.,* 2009, 4272-4274.

14 Y. Yang, Q. Liu, Y. Shi, Z. Song, *Eur. J. Med. Chem.,* 2014, **84**, 1-7.

15 S. Z. Ferreira, H. C. Carneiro, H. A. Lara, R. B. Alves, J. M. Resende, H. A. Oliveria, L. M. Silva, D. A. Santos, R. P. Freitas. *ACS. Med. Chem. Lett*., 2015, **6**, 271-273.

16 L. Zheng, G. Su, J. Ren, L. Gu, L. You, M. Zhao, *J. Agric. Food Chem.,* 2012, **60**, 5431-5437.

17 a) F. Tahoori, S. Balalaie, R. Sheikhnejad, M. Sadjadi, P. Boloori, *Amino Acids* 2014, **46**, 1033-1046; b) F. Tahoori, R. Sheikhnejad, S. Balalaie, M. Sajadi *Amino Acids* 2013, **45**, 975-981; c) Z. Rezaee, S. A. Arabanian, S. Balalaie, A. Ahmadiani, L. Khalaj, S. Nasoohi, *J. Pept. Sci*., 2001, **18**, 92-96.

18 R. Maggi, F. Bigi, S. Carloni, A. Mazzacani, G. Sartori, *Green Chem.,* 2001, 173-174.

19 C. Sun, C. Peng, J. Wang, Q. Wang, W. Liu, H. Zhou, C. Yang, *Heterocycles* 2013, **87**, 1711-1726.

20 C, L. Wong, K. Cheng, F. Chen, *Food. Chem*., 2006, **97**, 705-711.

21 Sharma, O., Bhat, K. (2009) DPPH antioxidant assay revisited. *Food. Chem. 113*, 1202-1205.

22 J. Jørgensen, D, C. Wasington, P. R. Murray, E. J. Baron, J, H. Jørgensen, M, A. Pfaller, F, C. Yolken, R, H. Yolken, *Am. Soc. Microbiol*., 2007, **9**, 1152-1172.

23 C. J. Kelley, R. C. Harruff, M. Carmack, *J. Org. Chem.,* 1976, **41**, 449-55.

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Graphical Abstract

Peptides *N*-Connected to Hydroxycoumarin and cinnamic acid derivatives: Synthesis and Fluorescence Spectroscopic, Antioxidant and Antimicrobial Properties

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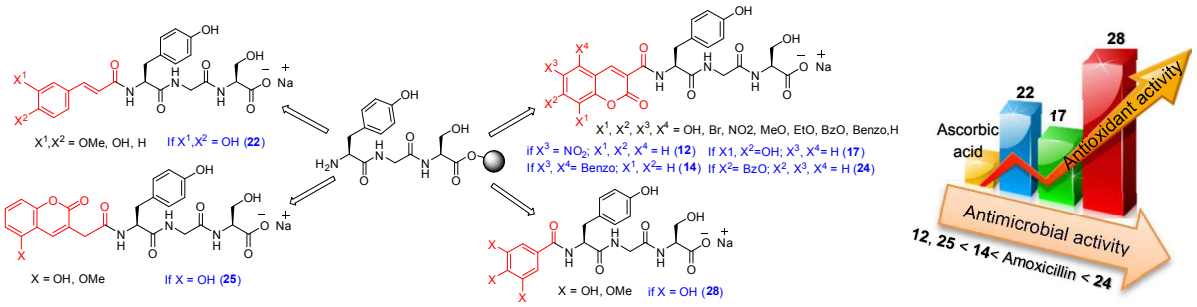

In this study, we developed an efficient synthesis of tripeptide Tyr-Gly-Ser and a series of conjugations to coumarin, cinnamic and gallic acid and their antioxidant and antimicrobial activities were investigated. The first application of BBr_3 as demethylating agent in synthesis of *N*-connecting peptide in solid phase peptide synthesis was reported. The activity results showed that, conjugated tripeptides 7,8-dihydroxycoumarin-tripeptide (**17**), caffeic acid- tripeptide (**22**) and gallic acid- tripeptide (**28**) were found to be superior to ascorbic acid with respect to their antioxidant activity, and **12**, **14**, **24**, and **25** exhibited the most antimicrobial activity in the series compared to amoxicillin. Additionally, fluorescence spectra of these conjugated peptides was investigated.