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α-Peptide/β-sulfonamidopeptide hybrids: Analogs of the chemotactic agent for-Met-Leu-Phe-OMe

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Abstract—In order to gain information on the activity shown by α-peptide/β-sulfonamidopeptide hybrid analogs of the potent chemotactic agent fMLF-OMe, a structure–activity study is reported on *N*-Boc- and *N*-formyl tripeptide models containing an aminoalkanesulfonic acid as central residue. Directed migration (chemotaxis), superoxide anion production, and lysozyme release have been measured. The biochemical functions and the conformational properties of the new compounds are discussed and related to previously studied models containing β-residues.

1. Introduction

Among the promising surrogates which can be considered for the replacement of the native amide bond of peptides, a particular place should be reserved for SO_2-NH. This junction possesses chemical and physicochemical properties, some of them particularly useful to overcome the well-known drawbacks which make native peptides hardly useful as therapeutic agents. Sulfonamides are metabolically stable, can effectively interact through hydrogen bond formation, adopt preferential secondary structures, and possess a tetrahedral sulfur atom mimicking the intermediate formed during the process of amide-bond hydrolysis.[1] Unfortunately, the intrinsic chemical lability of the sulfonamido-α-peptides, which are the closest sulfur analogs of natural peptides, prevents the exploitation of the benefits associated with this bioisosteric replacement.[2,3]

As viable alternative to sulfonamido-α-peptides the structurally related family of sulfonamido-β-peptides has been developed. These are peptidomimetics structurally related to the well-known class of β-peptides[4–6] and are based on the synthesis of β-aminoethanesulfonic acids as monomeric units and the study of the corresponding oligomers.[1,7] The high stability, the possibility of mono- and disubstitution on the β-skeleton as well as the physicochemical properties of the sulfonamide junction continue to stimulate interest on these peptidomimetics and on the related hybrid oligomers made up of both α-aminocarboxylic- and β-aminosulfonic-acids.

In this context, we reported previously[8,9] studies on the biochemical consequences of the introduction of a taurine (Tau, β-aminoethanesulfonic acid) into the peptide backbone of the potent chemotactic agent *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine methyl ester (for-Met-Leu-Phe-OMe; fMLF-OMe)[10–12] in place of the native central L-leucine. This structural modification was carried out as part of a research program, focused on fMLF-OMe analogs containing ω-amino acids at the central position, in order to investigate the structural and biological properties of such derivatives.[13] A detailed analysis of the role of the single residues on the activity of formyl peptide chemoattractants has been previously reported by Freer et al.[14] Data indicate that the alteration at the central position with residues bearing lipophilic side chains is well tolerated.[12] In accordance with these findings (see also Table V of Ref. 12) we found that the introduction of a short spacer such as –HN-CH_2-CH_2-CO– (i.e., β-Ala) generated the completely

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inactive chemotactic peptide for-Met-βAla-Phe-OMe.[9a] On the contrary and quite unexpectedly, the analogous structural modification performed by introducing as short spacer the sulfonyl analog of βAla (i.e.: Tau) gave rise to the highly active chemotactic peptide for-Met-Tau-Phe-OMe.[9a] Different sequences of the hybrid pseudopeptides, in which the Tau was located at the N-terminal position, did not generate active analogs.[8]

By taking into account the relevance of the activation of the formyl peptide receptor (FPR) in both antibacterial host defense and tissue repair processes[15,16] and in view of the continuing interest on β-sulfonamidopeptides,[1,17–21] we report here a study on a new group of α-peptide/β-sulfonamidopeptide hybrids, specifically designed to examine the role of the central sulfonamide junction on the structural properties and biological activities of chemotactic *N*-formyltripeptides.

2. Results and discussion

We have synthesized the Boc (**4a–f**) and formyl (**5a–f**) pseudotripeptides (Table 1), which are sulfonamide analogs of fMLF. In the models **4a–5a** and **4b–5b**, an L-alanine has been introduced in place of the N-terminal Met and C-terminal Phe residues, respectively. The analogs **4c** and **5c** contain palindromic sequence of fMLF, where the positions of the terminal residues Met and Phe are interchanged. In compounds **4d–5d** and **4e–5e**, the modification has been focused on the alteration of the chemical and physicochemical properties of the central junctions; thus, the CO–NH between the Met and Tau, and the SO_2–NH between the Tau and Phe have been replaced by the corresponding CO-N(Me) and SO_2-N(Me) bonds. Finally, in compounds **4f–5f** the Tau residue is substituted by its higher homolog, the 3-aminopropanesulfonic acid (homotaurine; HTau).

Table 1.

R-Ala-Tau-Phe-OMe (**a**)	R-Met-Tau-MePhe-OMe (**d**)
R-Met-Tau-Ala-OMe (**b**)	R-Met-MeTau-Phe-OMe (**e**)
R-Phe-Tau-Met-OMe (**c**)	R-Met-HTau-Phe-OMe (**f**)

4a–f: R = Boc; **5a–f**: R = for

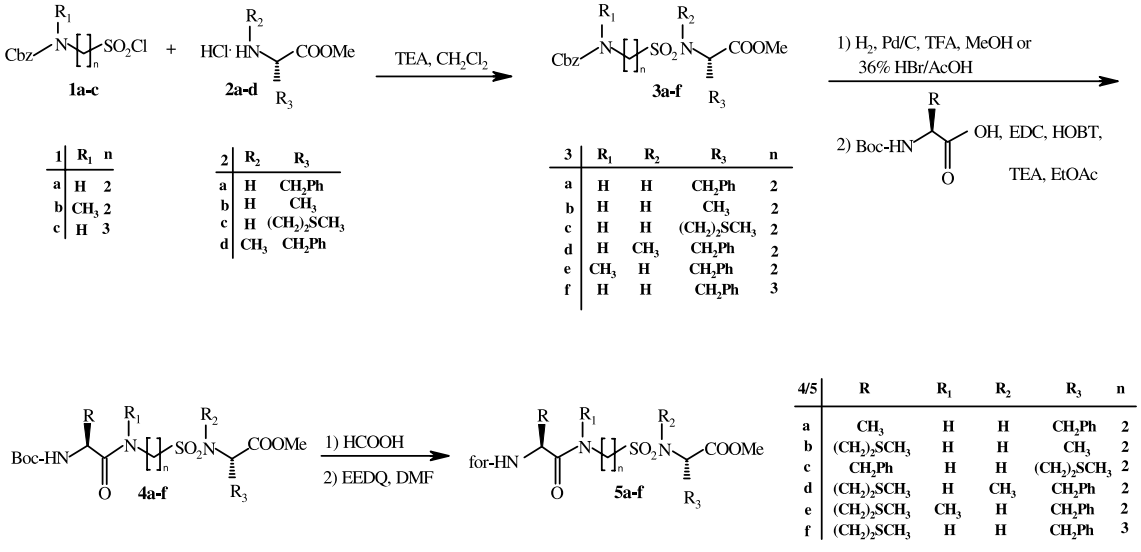
2.1. Chemistry

The synthesis of the *N*-Boc pseudotripeptides **4a–f** and the corresponding *N*-formyl analogs **5a–f** was performed according to Scheme 1. The Cbz-amino-alkanesulfonyl chlorides **1a–c** were obtained from taurine, *N*-methyl-taurine, and homotaurine, respectively, which were first N-protected by reaction with benzylchloroformate and then converted into the respective sulfonylchlorides by treatment with phosphorus pentachloride. This latter reaction, when performed on the homotaurine derivative Cbz-NH-$(CH_2)_3$-SO_3H, afforded, in addition to the desired sulfonyl chloride **1c**, the corresponding *N*-benzyloxycarbonyl-isothiazolidine-1,1-dioxide (*N*-Cbz-γ-sultam)[22] derived from the concurrent intramolecular cyclization reaction.

Coupling of the sulfonyl chlorides **1a–c** with the required amino acid methyl esters **2a–d**, by adopting the EDC/HOBT procedure, afforded the pseudodipeptides **3a–f**. N-deprotection followed by coupling with the *N*-Boc-protected amino acids gave the pseudotripeptides **4a–f**. A direct transformation of the *N*-Boc derivatives **4a–f** into the corresponding *N*-formyl analogs **5a–f** was performed by following the procedure of Lajoie and Kraus.[23]

2.2. Solution conformation

In order to investigate the conformations of formyl pseudotripeptides, the involvement of the NH groups in intramolecular hydrogen bonding for analogs **5a–f** was probed using ^{1}H NMR solvent-induced chemical shifts. In Figure 1, the chemical shift dependence of



1	R_1	n
a	H	2
b	CH_3	2
c	H	3

2	R_2	R_3
a	H	CH_2Ph
b	H	CH_3
c	H	$(CH_2)_2SCH_3$
d	CH_3	CH_2Ph

3	R_1	R_2	R_3	n
a	H	H	CH_2Ph	2
b	H	H	CH_3	2
c	H	H	$(CH_2)_2SCH_3$	2
d	H	CH_3	CH_2Ph	2
e	CH_3	H	CH_2Ph	2
f	H	H	CH_2Ph	3

4/5	R	R_1	R_2	R_3	n
a	CH_3	H	H	CH_2Ph	2
b	$(CH_2)_2SCH_3$	H	H	CH_3	2
c	CH_2Ph	H	H	$(CH_2)_2SCH_3$	2
d	$(CH_2)_2SCH_3$	H	CH_3	CH_2Ph	2
e	$(CH_2)_2SCH_3$	CH_3	H	CH_2Ph	2
f	$(CH_2)_2SCH_3$	H	H	CH_2Ph	3

Scheme 1. Synthesis of pseudotripeptides **4a–f** and **5a–f**.

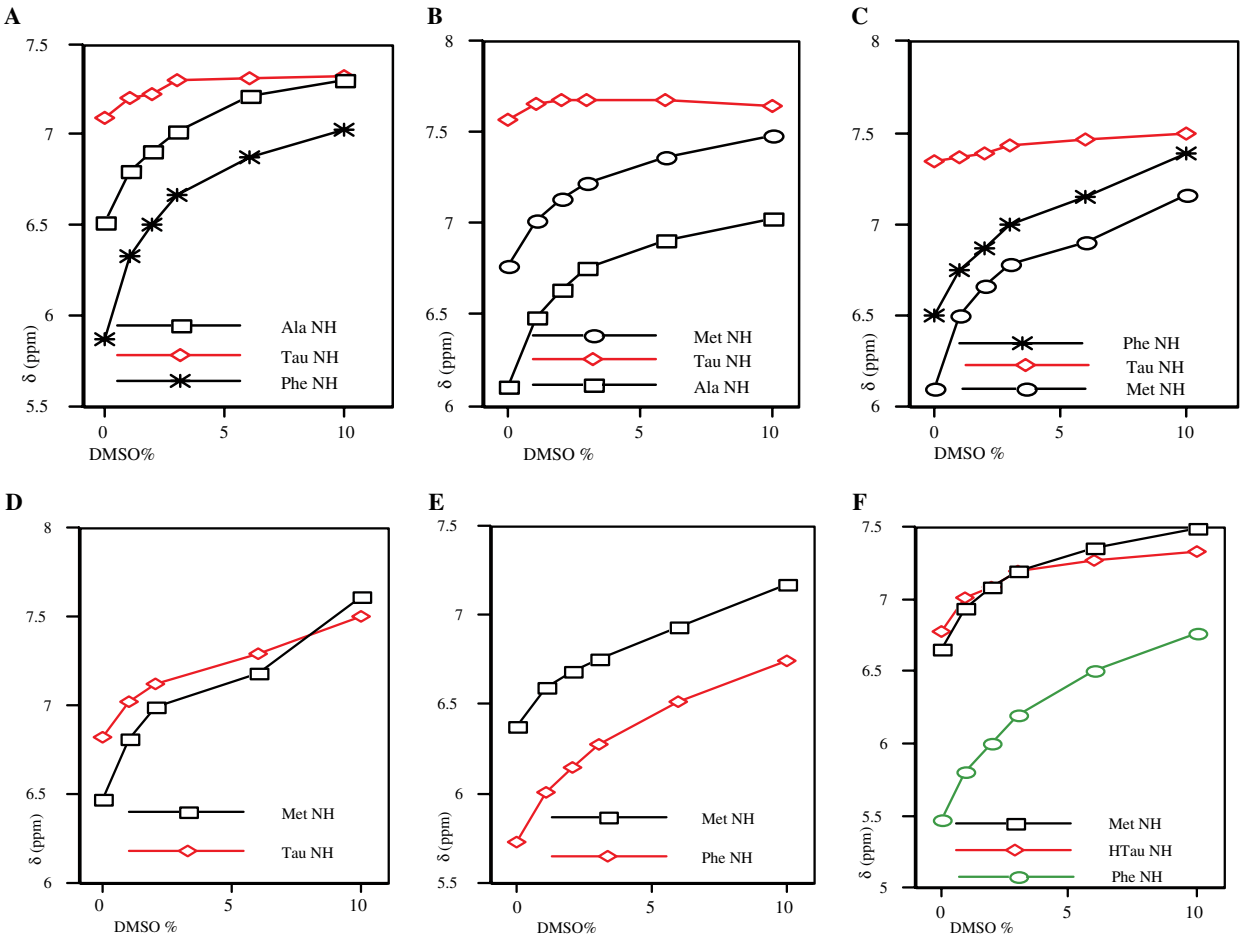


Figure 1. Plots of NH proton chemical shifts in the ^{1}H NMR spectra of the tripeptide derivatives **5a** (A), **5b** (B), **5c** (C), **5d** (D), **5e** (E), and **5f** (F) as a function of increasing amounts of DMSO-d_6 (v/v) added to $CDCl_3$ solution (peptide concentration 10 mM).

Table 2. ^{1}H NMR solvent accessibility of peptide NH groups: differences ($\Delta\delta$, ppm) between NH proton chemical shift values observed in a $CDCl_3$ solution containing 10% DMSO-d_6 and in neat $CDCl_3$

Compound	N-terminal NH	Central NH	C-terminal NH
HCO-Ala-Tau-Phe-OMe (**5a**)	0.79	0.23	1.15
HCO-Met-Tau-Ala-OMe (**5b**)	0.71	0.07	0.91
HCO-Phe-Tau-Met-OMe (**5c**)	0.89	0.16	1.06
HCO-Met-Tau-(*N*-Me)Phe-OMe (**5d**)	1.14	0.68	
HCO-Met-(*N*-Me)Tau-Phe-OMe (**5e**)	0.79		1.01
HCO-Met-NH$(CH_2)_3$-SO_2-Phe-OMe (**5f**)	0.84	0.55	1.30

the NH resonances of **5a–f** as a function of increasing DMSO-d_6 concentration in $CDCl_3$ solution (10 mM) is shown. The resulting solvent exposure of the NH groups of **5a–f**, expressed as the difference ($\Delta\delta$, ppm) between the NH chemical shift values observed in a $CDCl_3$ solution containing 10% DMSO and in neat $CDCl_3$, is reported in Table 2.

The results of the solvent perturbation experiments clearly show that in the three models **5a–c** the central CO-NH presents a pronounced solvent inaccessibility; in particular, the Tau NH of the tripeptide **5b** is practically unaffected by the increase of the DMSO-d_6 concentration ($\Delta\delta$ = 0.07 ppm). On the contrary, the NH groups of the external Ala, Met, and Phe of the same group of analogs interact efficiently with the solvent especially when located at the C-terminal position ($\Delta\delta$ values ranging from 0.91 to 1.15 ppm). This behavior suggests that, at variance with the NH groups of external residues, the central amide Tau NH of **5a–c** is involved in an intramolecular hydrogen bond. A different behavior is observed in the case of the other three peptides **5d–f** (Figs. 1D–F and Table 2) where the NH groups of all the residues show appreciable solvent dependence of chemical shift with only a moderate inaccessibility exhibited by the HTau NH group of **5f**. Although the NMR titration experiments only provide an identification of potentially intramolecularly hydrogen bonded NH groups, without giving any information on the nature of the acceptors, the present data indicate

that, at variance with the three analogs **5d–f**, characterized by a large extent of unfolded conformations, the analogs **5a–c** appear to be in a folded conformation involving the Tau NH as hydrogen bond donor. In this context, it is interesting to note that an analogous result was obtained when the ^{1}H NMR solvent perturbation experiments were performed on small linear α/β-hybrid peptides containing a β-Ala residue.[9b] Here the recurrent behavior was the solvent inaccessibility shown by the β-Ala CO–NH group and this, on the basis of literature data, was interpreted as indicative of the occurrence of locally folded conformers centered at such residue through a six-membered ring (C_6 conformation).[24]

2.3. Biological activity

The biological activities, namely, the direct migration (chemotaxis), superoxide anion production, and lysozyme release have been determined on human neutrophils for all the synthesized compounds. The results of the chemotactic activity, expressed as chemotactic index (C.I.) (Section 4.7.2), are summarized in Figure 2. In Figure 2A, the activity of the tripeptides **4a–c** and **5a–c** is reported.

The data suggest that these six compounds are able to stimulate only moderate chemotactic activity (partial agonists) with the two *N*-formyl derivatives **5b** and **5c** more active than **5a** and clearly more active than the three *N*-Boc analogs **4a–c**. However, all these compounds are significantly less active than the previously studied analogs Boc-Met-Tau-Phe-OMe and for-Met-Tau-Phe-OMe which reach, at physiological concentration (10^{-9} M), C.I. values of 1.0 ca. When the activity of the three *N*-formyl derivatives **5a–c** is considered, it can be seen that **5b**, which maintains the Met at the first position and contains the C-terminal Ala, exhibits the highest activity, while **5a**, where the Ala replaces the Met, is the least active. This indicates that in these Tau-containing pseudopeptides the lipophilic side chain at the N-terminal position plays an important role similar to that found in the usual α-peptidic fMLF-OMe analogs. This is evident by the higher activity shown by for-Phe-Tau-Met-OMe (**5c**) as compared with that for-Ala-Tau-Phe-OMe (**5a**) as well as by the behavior of Boc-Ala-Tau-Phe-OMe (**4a**) which has the lowest activity among the peptides reported in Figure 2A. Figure 2B shows the chemotactic activity of the *N*-Boc pseudotripeptides **4d–f** and of the corresponding *N*-formyl derivatives **5d–f**. For this group of peptides, as against those shown in Figure 2A, the chemotactic activity exhibited by Boc derivatives is significantly different from that exhibited by the respective formyl derivatives. Compounds **4d–f** demonstrate marginal activity (the peaks of C.I. do not exceed 0.35 at a concentration of 10^{-9} M), while all the three *N*-formyl analogs are strong agonists which, in the case of for-Met-(N-Me)Tau-Phe-

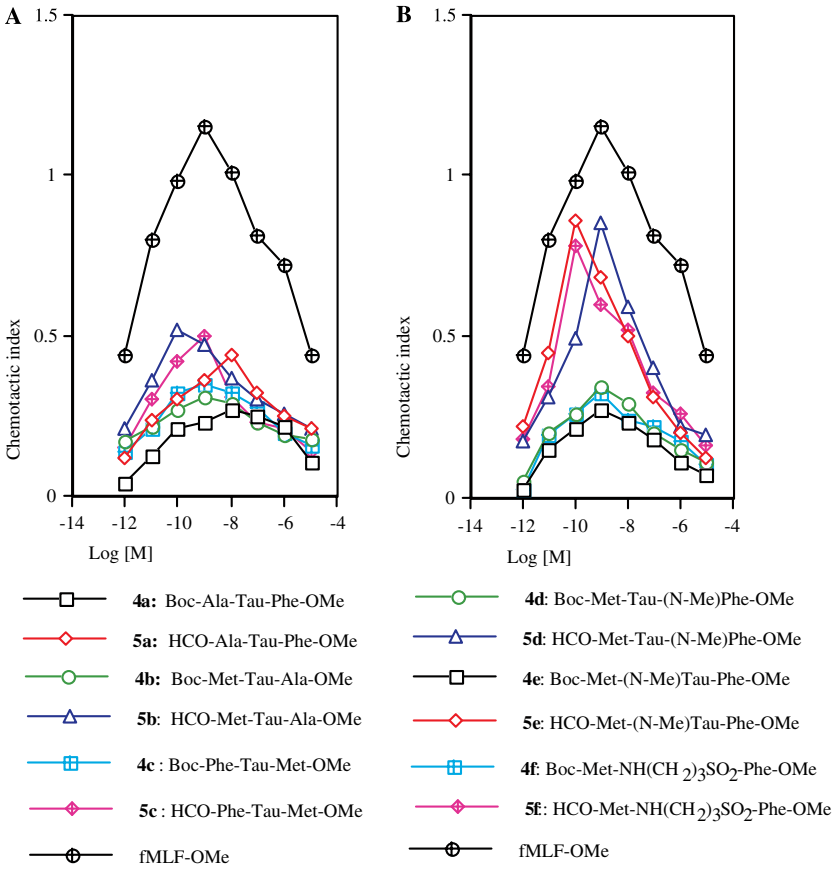


Figure 2. Chemotactic activity of tripeptide derivatives **4a–f** and **5a–f** expressed as chemotactic index (see Section 4.7.2).

OMe **5d**, reach a maximum C.I. value of 0.85 at a concentration of 10^{-10} M.

Previous papers report the biological consequences of the alteration or elimination of the NH groups at position 2 and 3 of the parent peptide fMLF-OMe.[25,26] The results, performed on analogs containing *N*-methyl amide or ester bonds in place of the native CO-NH, clearly demonstrate the importance for the activity of the presence of the hydrogen bond donor NH groups at the Met-Leu and Leu-Phe junctions. In the present study, we have examined the two analogs **5d** and **5e** containing (*N*-Me)Phe and (*N*-Me)Tau at position 2 and 3, respectively. These derivatives exhibit good activity (Fig. 2B) which is, however, lower than that shown by the corresponding previously studied non-N-methylated pseudopeptidic analog for-Met-Tau-Phe-OMe.[9a] Thus, the consequences of the alkylation of NH-groups in sulfonamidopeptides are distinctly different from non-sulfonamide analogs, where the elimination of the protic bonds at positions 2 and 3 leads to practically inactive chemotactic peptides.[25,26]

As reported in Figure 2B, significant chemotactic activity (0.80 C.I. peak at a concentration of 10^{-10} M) is found for the peptide for-Met-HTau-Phe-OMe (**5f**) containing a γ-aminopropanesulfonic acid (HTau) at the central position of the backbone. A comparable value of activity, at the same concentration, is shown by the previously studied sulfonamidopeptide for-Met-Tau-Phe-OMe.[9a] In contrast, similar structure modifications, involving the residues of β-Ala and γ-Abu, cause inactivity or significant loss of activity, respectively.[9a,13] Therefore, both the Tau and HTau residues can be incorporated at the central position of fMLF-OMe generating pseudopeptide analogs which show either high or significant chemotactic activity in comparison to the corresponding carboxamido models.

Concerning the superoxide anion production (Fig. 3B) all the tested derivatives, except the *N*-formyl derivatives **5d** and **5e**, resulted to be practically inactive. For the two analogs **5d** and **5e**, both characterized by the presence of a *N*-Me residue (Table 1), an agonistic activity was detected only at high concentrations (10^{-4}–10^{-5} M) (Fig. 3B) and this indicates weak efficacy by the peptides which can be considered partial agonists with regard to O_2^- production. The corresponding *N*-Boc derivatives, **4d** and **4e**, also reported in Figure 3B, do not show activity.

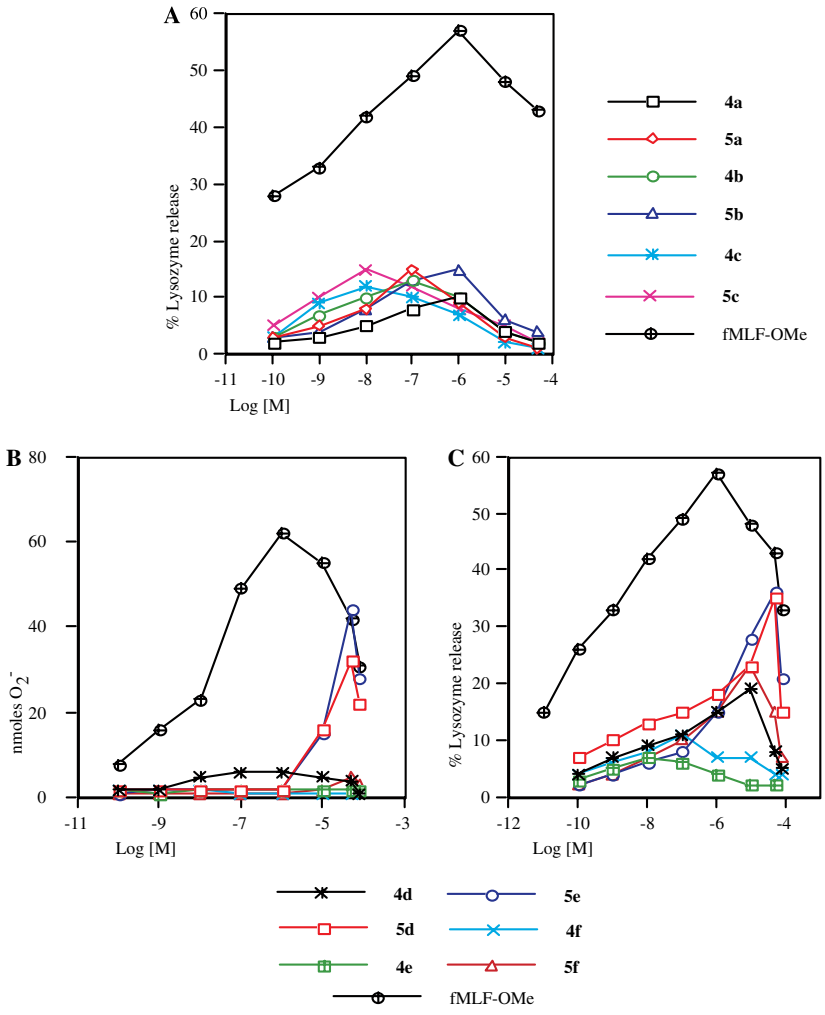


Figure 3. Release of neutrophil granule enzymes evaluated by determining lysozyme activity induced by **4a–c** and **5a–c** (A) and by **4d–f** and **5d–f** (C). Superoxide anion (O_2^-) production triggered by **4d–f** and **5d–f** (B) (see Sections 4.7.3 and 4.7.4.).

As shown in Figure 3A, the activity as secretagogue agents of the *N*-Boc and *N*-for peptides, characterized by the presence of Ala at the external positions (**4a**–**b** and **5a**–**b**) or by the Met/Phe interchange (**4c**–**5c**) (see Table 1), is very low. In Figure 3C, the enzyme release activity is reported for the group of models **4d**–**f** and **5d**–**f** which present, as compared with peptides reported in Figure 3A, only minor modifications of the Met-Tau-Phe side-chain sequence (see Table 1). Here, similar to the superoxide anion production, an activity comparable to that of the fMLP-OMe is shown only at high concentration by the two analogs **5d** and **5e** characterized by MePhe or MeTau replacements.

It can be recalled that the consequences of the alteration of the native sequence of amino acids on the lysozyme release, here examined in the model for-Phe-Tau-Met-OMe (**5c**), have been previously evaluated by Bonora et al.[27] while studying a group of retroisomers of fMLF. The authors found that for-Phe-Leu-Met-NH_2 was approximately 100-fold less active than the parent for-Met-Leu-Phe-NH_2. The high fall in activity exhibited by this retroisomer parallels the result here found for the related pseudopeptide **5c** (see Fig. 3A). Thus, the maintenance of the native side chain sequence at the N- and C-terminal positions is confirmed as an important factor in the receptor recognition for usual peptides as well as for the here studied sulfonamido analogs.

3. Conclusion

In conclusion, we have synthesized and studied a series of pseudopeptidic fMLF analogs characterized by the presence of a central sulfonamide junction and different alterations of both the backbone and the native side-chain sequence. Results show that, similar to the literature findings on related classical peptide analogs,[12,14] the presence in sulfonamide models of the for-Met at the N-terminal position is of primary importance for optimal biological activity. On the contrary, the N-methylation of the Tau and Phe NH groups, as well as the homologation of the central β-residue (see compounds **5d**–**f**), can be performed with only moderate decrease of the chemotactic activity shown by for-Met-Tau-Phe-OMe.[9a] Results on lysozyme release and superoxide anion production (Fig. 3) show a clear negative effect of the central sulfonamide bond on the two main biological functions associated with chemotaxis. Analogs of fMLF containing a central β-sulfonamido residue seem then promising and flexible models for designing selective agonists, with particular reference to chemoattractants devoid of superoxide anion production.[28]

Taken together the reported results indicate that the sulfonamide junction, due to its high polar character and conformational properties, when located at the central position of fMLF analogs to replace the native Leu (residue), produces a profound alteration of the established requirements for optimal ligand-receptor interaction. The resulting properties, together with the observed capability to discriminate among the biological activities exerted by the fMLF, might render the here-described compounds a very useful tool for studying the signal transduction mechanisms of the fMLF receptors and their different subtypes.[28]

4. Experimental

4.1. General methods

Melting points were determined with a Büchi B 540 apparatus and are uncorrected. Optical rotations were taken at 20 °C with a Schmidt-Haensch Polartronic D polarimeter (1 dm cell, *c* 1.0 in $CHCl_3$, unless otherwise specified). IR spectra were recorded in 1% $CHCl_3$ (unless otherwise specified) solution employing a Perkin-Elmer FT-IR Spectrum 1000 spectrometer. ^1H NMR spectra were determined in $CDCl_3$ solution with a Bruker AM 400 spectrometer and chemical shifts were indirectly referred to TMS. Thin-layer and preparative layer chromatographies were performed on silica gel Merck 60 F_{254} plates. The drying agent was sodium sulfate. Elemental analyses were performed in the laboratories of the Servizio Microanalisi del CNR, Area della Ricerca di Roma, Montelibretti, Italy, and were within ±0.4% of the theoretical values. The abbreviations used are as follows: Boc, *tert*-butyloxycarbonyl; EEDQ, ethyl 2-ethoxy-1,2-dihydro-1-quinolinecarboxylate; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; KRPG, Krebs–Ringer-phosphate containing 0.1% w/v D-glucose; NMM, *N*-methylmorpholine; DMF, dimethylformamide; TEA, triethylamine.

4.2. General procedure for the preparation of Cbz-amino-alkanesulfonyl chlorides 1a–c

A solution of the amino-alkanesulfonic acid (1.0 mmol) in H_2O (10 mL) was adjusted to pH 8.5 by using an aqueous solution of 1 M NaOH. Under vigorous stirring, benzylchloroformate (1.1 mmol) was added in five portions at room temperature, maintaining the pH at the above value by addition of small amount of NaOH, then the reaction mixture was stirred overnight. After dilution with H_2O (10 mL), the aqueous phase was washed with Et_2O (2× 10 mL) and evaporated. The crude solid residue was coevaporated three times with toluene and dried under high vacuum over P_2O_5 overnight. To a suspension of the crude sodium sulfonate salt in anhydrous Et_2O (10 mL), PCl_5 (1.5 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was washed with water and the organic phase dried and evaporated. Compounds **1a**[29] and **1b**[30] were obtained as colorless oil which solidified by trituration with hexane.

4.3. General procedure for the preparation of pseudodipeptides 3a–f

To an ice-cooled mixture containing the Cbz-amino-alkanesulfonyl chloride **1a**–**c** (1.0 mmol) and the amino acid methyl ester hydrochlorides **2a**–**d** (1.0 mmol) in dry CH_2Cl_2 (8 mL), a solution of TEA (2.0 mmol) in dry CH_2Cl_2 (8 mL) was added dropwise under nitrogen.

The resulting suspension was stirred overnight allowing to warm to room temperature. After dilution with CH_2Cl_2 (25 mL), the mixture was washed with 1 M HCl (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL), and brine (10 mL). The organic phase was dried and evaporated under reduced pressure.

4.3.1. Cbz-tauryl-L-alanine methyl ester (3b). From Cbz-tauryl chloride **1a** (0.800 g, 2.88 mmol) and L-alanine methyl ester hydrochloride (**2b**) (0.400 g). Pale yellow oil, used in the next step without further purification. (0.694 g, 70%). $[\alpha]_D$ −45°. IR *v*: 3448, 3367, 2957, 1717 cm^{-1}. ^1H NMR δ: 1.35 (3H, d, J = 6.4 Hz, Ala CH_3), 3.14 (2H, m, Tau α-CH_2), 3.62 (2H, m, Tau β-CH_2), 3.70 (3H, s, $COOCH_3$), 4.13 (1H, m, Ala α-CH), 5.05 (2H, s, CH_2Ph), 5.38 (1H, d, J = 9.4 Hz, Ala NH), 5.35 (1H, poorly resolved t, Tau NH), 7.28 (5H, s, aromatics). Anal. Calcd for $C_{14}H_{20}N_2O_6S$: C, 48.83; H, 5.85; N, 8.13. Found: C, 48.58; H, 5.71; N, 8.15.

4.3.2. Cbz-tauryl-L-methionine methyl ester (3c). From Cbz-tauryl chloride **1a** (0.500 g, 1.80 mmol) and L-methionine methyl ester hydrochloride **2c** (0.360 g). The crude product was triturated with hexane. Pale yellow solid. (0.60 g, 82%). $[\alpha]_D$ −37°. IR *v*: 3448, 2957, 1718 cm^{-1}. ^1H NMR δ: 1.75–2.18 (2H, m, Met β-CH_2), 2.03 (3H, s, S-CH_3), 2.51 (2H, m, Tau α-CH_2), 3.13 (2H, m, Tau β-CH_2), 3.63 (2H, m, Met γ-CH_2), 3.70 (3H, s, $COOCH_3$), 4.28 (1H, m, Met α-CH), 5.03 (2H, s, CH_2Ph), 5.30 (1H, d, J = 9.6 Hz, Met NH), 5.40 (1H, poorly resolved t, Tau NH), 7.14 (5H, s, aromatics). Anal. Calcd for $C_{16}H_{24}N_2O_6S_2$: C, 47.51; H, 5.98; N, 6.93. Found: C, 47.72; H, 6.04; N, 6.81.

4.3.3. Cbz-tauryl-(*N*-methyl)-L-phenylalanine methyl ester (3d). From Cbz-tauryl chloride **1a** (0.233 g, 0.84 mmol) and (*N*-methyl)-L-phenylalanine methyl ester hydrochloride **2d** (0.163 g). Silica gel flash chromatography ($CHCl_3$) gave the pure product as a pale yellow oil. (0.298 g, 82%). $[\alpha]_D$ −15°. IR *v*: 3688, 3446, 2955, 2406, 1718 cm^{-1}. ^1H NMR δ: 2.65 (2H, m, Tau α-CH_2), 2.74–3.00 (2H, m, Phe β-CH_2), 2.68 (3H, s, N-CH_3), 3.24–3.45 (2H, m, Tau β-CH_2), 3.76 (3H, s, $COOCH_3$), 4.86 (1H, m, Phe α-CH), 5.10 (2H, s, CH_2Ph), 5.35 (1H, br s, Tau NH), 7.14–7.40 (10H, m, aromatics). Anal. Calcd for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45. Found: C, 58.15; H, 5.92; N, 6.40.

4.3.4. Cbz-(*N*-methyl)tauryl-L-phenylalanine methyl ester (3e). From Cbz-(*N*-methyl)tauryl chloride **1b** (0.361 g, 1.24 mmol) and L-phenylalanine methyl ester hydrochloride **2a** (0.267 g). Pale yellow oil used in the next step without further purification. (0.390 g, 72%). $[\alpha]_D$ −10°. IR *v*: 3550, 3360, 2960, 1743, 1697 cm^{-1}. ^1H NMR δ: 2.70–3.12 (4H, m, Tau α-CH_2 and Phe β-CH_2), 2.90 (3H, s, N-CH_3), 3.42 (2H, m, Tau β-CH_2), 3.62 (3H, s, $COOCH_3$), 4.31 (1H, m, Phe α-CH), 5.02 (2H, s, CH_2Ph), 5.41 (1H, d, J = 9.6 Hz, Phe NH), 7.01–7.48 (10H, m, aromatics). Anal. Calcd for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45. Found: C, 58.02; H, 6.18; N, 6.77.

4.3.5. Cbz-homotauryl-L-phenylalanine methyl ester (3f). From Cbz-homotauryl chloride **1c** (0.660 g, 2.26 mmol) and L-phenylalanine methyl ester hydrochloride **2a** (0.478 g). Silica gel flash chromatography ($CHCl_3$) gave the pure product as colorless oil. (0.501 g, 51%). $[\alpha]_D$ +7°. IR *v*: 3445, 2959, 1740, 1718 cm^{-1}. ^1H NMR δ: 1.94 (2H, m, HTau β-CH_2), 2.82 (2H, m, HTau α-CH_2), 3.05 and 3.17 (4H, two m, Phe β-CH_2 and HTau γ-CH_2), 3.92 (3H, s, $COOCH_3$), 4.43 (1H, m, Phe α-CH), 4.95–5.06 (2H, m, Phe and HTau NH), 5.24 (2H, s, CH_2Ph), 7.23–7.58 (10H, m, aromatics). Anal. Calcd for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45. Found: C, 57.98; H, 6.22; N, 6.17.

4.4. General procedure for the N-deprotection of pseudodipeptides 3a–f

Method a (HBr/AcOH): the N-protected pseudodipeptide (1.0 mmol) was dissolved in a solution of 36% HBr in acetic acid (3.0 mL). After 15 min at room temperature, the reaction mixture was evaporated to dryness without heating. The residue was repeatedly coevaporated with anhydrous Et_2O and then dried under high vacuum overnight.

Method b (catalytic hydrogenation): a solution of the N-protected pseudodipeptide (1.0 mmol) and trifluoroacetic acid (1.2 mmol) in MeOH (10 mL) was flushed with an H_2 stream in the presence of 10% palladium on charcoal at room temperature until the reaction was complete (TLC). The mixture was filtered on Celite and the filtrate was evaporated to dryness under reduced pressure. The oily residue was repeatedly coevaporated with anhydrous diethyl ether to remove excess TFA and then kept under high vacuum overnight.

4.5. General procedure for the preparation of pseudotripeptides 4a–f. Carbodiimide coupling

To an ice-cooled mixture containing the required Boc-protected amino acid (1.0 mmol), the pseudodipeptide salt **3a–f** (1.0 mmol), HOBt (1.2 mmol), and TEA (1.2 mmol) in anhydrous ethyl acetate (6 mL), EDC (1.0 mmol) was added and the reaction mixture was allowed to warm to room temperature. After 12 h, the reaction mixture was diluted with EtOAc (20 mL) and washed with 1M $KHSO_4$ (2 × 15 mL), saturated aqueous $NaHCO_3$ (2 × 15 mL), and brine (15 mL). The organic phase was dried and evaporated under reduced pressure.

4.5.1. Boc-L-alanyl-tauryl-L-phenylalanine methyl ester (4a). Cbz-tauryl-L-phenylalanine methyl ester[31] **3a** (0.441 g, 1.04 mmol) was N-deprotected according to the above general procedure (*method b*). The dipeptide, as trifluoroacetate salt (white solid), was acylated with Boc-L-alanine (0.197 g, 1.04 mmol) according to the carbodiimide coupling general procedure. Silica gel flash chromatography (CH_2Cl_2/MeOH 98:2) gave the pure product as a colorless oil (0.414 g, 87%). $[\alpha]_D$ −49°. IR *v*: 3436, 3017, 1742, 1682 cm^{-1}. ^1H NMR δ: 1.26 (3H, d, J = 7.2 Hz, Ala CH_3), 1.36 [9H, s, $C(CH_3)_3$], 2.78 and 2.85 (2H, m, Tau α-CH_2), 2.95 and 3.10 (2H, A and B of on ABX, J = 5.1, 8.7 and 13.9 Hz, Phe

β-CH_2), 3.42 and 3.51 (2H, two m, Tau β-CH_2), 3.70 (3H, s, $COOCH_3$), 4.02 (1H, m, Ala α-CH), 4.34 (1H, m, Phe α-CH), 4.93 (1H, d, $J = 7.5$ Hz, Ala NH), 5.84 (1H, br s, Phe NH), 6.95 (1H, br s, Tau NH), 7.13–7.26 (5H, m, aromatics). Anal. Calcd for $C_{20}H_{31}N_3O_7S$: C, 52.50; H, 6.83; N, 9.18. Found: C, 52.37; H, 6.95; N, 9.08.

4.5.2. Boc-L-methionyl-tauryl-L-alanine methyl ester (4b). Cbz-tauryl-L-alanine methyl ester **3b** (0.413 g, 1.2 mmol) was N-deprotected according to the above general procedure (*method b*). The dipeptide, as trifluoroacetate salt (white solid), was acylated by Boc-L-methionine (0.300 g, 1.2 mmol) according to the carbodiimide coupling general procedure. Silica gel flash chromatography (CH_2Cl_2/MeOH 99:1) gave the pure product as a colorless oil (0.397 g, 75%). $[\alpha]_D$ −11°. IR *v*: 3428, 1732, 1690 cm^{-1}. ^1H NMR δ: 1.40 [9H, s, $C(CH_3)_3$], 1.55 (3H, d, $J = 8.2$ Hz, Ala CH_3), 1.80 and 1.93 (2H, two m, Met β-CH_2), 2.13 (3H, s, S-CH_3), 2.59 (2H, m, Met γ-CH_2), 3.28 (2H, m, Tau α-CH_2), 3.62 and 3.76 (2H, m, Tau β-CH_2), 3.82 (3H, s, $COOCH_3$), 4.19 (1H, m, Met α-CH), 4.32 (1H, m, Ala α-CH), 5.28 (1H, d, $J = 8.0$ Hz, Met NH), 6.19 (1H, d, $J = 7.4$ Hz, Ala NH), 7.56 (1H, poorly resolved t, Tau NH). Anal. Calcd for $C_{16}H_{31}N_3O_7S_2$: C, 43.52; H, 7.08; N, 9.52. Found: C, 43.22; H, 7.15; N, 9.41.

4.5.3. Boc-L-phenylalanyl-tauryl-L-methionine methyl ester (4c). Cbz-tauryl-L-methionine methyl ester **3c** (0.578 g, 1.43 mmol) was N-deprotected according to the above general procedure (*method a*). The dipeptide, as hydrobromide (white solid), was acylated by Boc-L-phenylalanine (0.380 g, 1.43 mmol) according to the carbodiimide coupling general procedure. Crystallization from diethyl ether/hexane gave the pure product as a white solid (0.547 g, 74%). $[\alpha]_D$ +21° (2% $CHCl_3$). Mp 95–97 °C. IR *v*: 3432, 3037, 1731, 1684 cm^{-1}. ^1H NMR δ: 1.45 [9H, s, $C(CH_3)_3$], 2.00–2.12 [4H, m, 1H of Met β-CH_2 and S-CH_3 (s at 2.10)], 2.18 (1H, m, 1H of Met β-CH_2), 2.66 (2H, m, Met β-CH_2), 3.07 and 3.17 (4H, two m, Phe β-CH_2 and Tau α-CH_2), 3.52 and 3.89 (2H, two m, Tau β-CH_2), 3.84 (3H, s, $COOCH_3$), 4.30 (1H, m, Phe α-CH), 4.35 (1H, m, Met α-CH), 5.11 (1H, d, $J = 6.8$ Hz, Phe NH), 6.14 (1H, d, $J = 8.1$ Hz, Met NH), 7.23–7.34 (5H, m, aromatics), 7.36 (1H, poorly resolved t, Tau NH). Anal. Calcd for $C_{23}H_{35}N_3O_7S_2 \cdot 0.5\ H_2O$: C, 50.17; H, 6.89; N, 7.98. Found: C, 50.28; H, 6.66; N, 7.95.

4.5.4. Boc-L-methionyl-tauryl-L-(N-methyl)phenylalanine methyl ester (4d). Cbz-tauryl-(*N*-methyl)-L-phenylalanine methyl ester **3d** (0.260 g, 0.60 mmol) was N-deprotected according to the above general procedure (*method b*). The dipeptide, as trifluoroacetate salt (white solid), was acylated by Boc-L-methionine (0.150 g, 0.60 mmol), according to the carbodiimide coupling general procedure. Colorless oil (0.280 g, 88%). $[\alpha]_D$ −14°. IR *v*: 3435, 3039, 1735 cm^{-1}. ^1H NMR δ: 1.45 [9H, s, $C(CH_3)_3$], 1.75 and 1.88 (2H, two m, Met β-CH_2), 2.09 (3H, s, S-CH_3), 2.48–2.63 (3H, m, Met γ-CH_2 and 1H of Tau α-CH_2), 2.70–2.93 [4H, m, 1H of Tau α-CH_2 and *N*-CH_3 (s at 2.91)], 2.95, and 3.36 (2H, AB of on ABX, $J = 5.5$, 10.2, and 14.2 Hz, Phe β-CH_2), 3.45 (2H, m, Tau β-CH_2), 3.79 (3H, s, $COOCH_3$), 4.22 (1H, m, Met α-CH), 4.86 (1H, m, Phe α-CH), 5.12 (1H, br s, Met NH), 6.68 (1H, br s, Tau NH), 7.14–7.26 (5H, m, aromatics). Anal. Calcd for $C_{23}H_{37}N_3O_7S_2$: C, 51.96; H, 7.01; N, 7.90. Found: C, 51.68; H, 6.93; N, 7.78.

4.5.5. Boc-L-methionyl-(*N*-methyl)tauryl-L-phenylalanine methyl ester (4e). Cbz-(*N*-methyl)tauryl-L-phenylalanine methyl ester **3e** (0.364 g, 0.84 mmol) was N-deprotected according to the above general procedure (*method b*). The dipeptide, as trifluoroacetate salt (white solid), was acylated by Boc-L-methionine (0.210 g, 0.84 mmol) according to the carbodiimide coupling general procedure. Silica gel flash chromatography ($CHCl_3$) gave the pure product as colorless oil. (0.366 g, 82%). $[\alpha]_D$ −30°. IR *v*: 3430, 3034, 1733, 1707, 1645 cm^{-1}. ^1H NMR δ: 1.42 [9H, s, $C(CH_3)_3$], 1.76 and 1.93 (2H, two m, Met β-CH_2), 2.11 (3H, s, S-CH_3), 2.54 (2H, m, Met γ-CH_2), 2.94–3.13 [7H, m, Phe β-CH_2, Tau α-CH_2 and N-CH_3 (s at 3.11)], 3.35 (1H, m, 1H of Tau β-CH_2), 3.75 (3H, s, $COOCH_3$), 4.13 (1H, m, 1H of Tau β-CH_2), 4.44 (1H, m, Met α-CH), 4.71 (1H, m, Phe α-CH), 5.33 (1H, d, $J = 6.7$ Hz, Met NH), 5.66 (1H, d, $J = 8.3$ Hz, Phe NH), 7.23–7.46 (5H, m, aromatics). Anal. Calcd for $C_{23}H_{37}N_3O_7S_2$: C, 51.96; H, 7.01; N, 7.90. Found: C, 52.04; H, 7.15; N, 7.82.

4.5.6. Boc-L-methionyl-homotauryl-L-phenylalanine methyl ester (4f). Cbz-homotauryl-L-phenylalanine methyl ester **3f** (0.148 g, 0.34 mmol) was N-deprotected according to the above general procedure (*method b*). The dipeptide, as trifluoroacetate salt (white solid), was acylated by Boc-L-methionine (0.086 g, 0.34 mmol) according to the carbodiimide coupling general procedure. Silica gel flash chromatography ($CHCl_3$/MeOH 98:2) gave the pure product as a pale yellow oil. (0.154 g, 85%). $[\alpha]_D$ −26°. IR *v*: 3343, 3039, 1676 cm^{-1}. ^1H NMR δ: 1.45 [9H, s, $C(CH_3)_3$], 1.82 (2H, m, HTau β-CH_2), 1.90 (1H, m, 1H of Met β-CH_2), 2.06 [4H, m, 1H of Met β-CH_2 and S-CH_3 (s at 2.11)], 2.53 (2H, m, Met γ-CH_2), 2.77 (2H, m, HTau α-CH_2), 3.00–3.39 (4H, three m, Phe β-CH_2 and HTau γ-CH_2), 3.78 (3H, s, $COOCH_3$), 4.19 (1H, m, Met α-CH), 4.40 (1H, m, Phe α-CH), 5.06 (1H, d, $J = 9.0$ Hz, Phe NH), 5.18 (1H, d, $J = 6.6$ Hz, Met NH), 6.52 (1H, poorly resolved t, HTau NH), 7.19–7.36 (5H, m, aromatics). Anal. Calcd for $C_{23}H_{37}N_3O_7S_2$: C, 51.96; H, 7.01; N, 7.90. Found: C, 51.74; H, 7.08; N, 7.59.

4.6. General procedure for preparation of the *N*-formyl derivatives 5a–f

The *N*-Boc derivative (1.0 mmol) was dissolved in formic acid (3 mL) and the mixture was allowed to stand at room temperature overnight. After removal of the excess formic acid under vacuum, the residue was dissolved in dry DMF (2 mL). EEDQ 97% (1.2 mmol) was added and the solution was stirred at room temperature for 24 h. Evaporation under reduced pressure afforded the crude product.

4.6.1. Formyl-L-alanyl-tauryl-L-phenylalanine methyl ester (5a). From Boc-L-alanyl-tauryl-L-phenylalanine methyl ester **4a** (0.283 g, 0.61 mmol). Silica gel flash

chromatography (CH_2Cl_2/MeOH 98:2) gave the pure product as a white foam. (0.188 g, 80%). $[\alpha]_D$ −10°. Mp 126–128 °C. IR *v*: 3412, 1742, 1673 cm^{-1}. ^1H NMR δ: 1. 35 (3H, d, *J* = 6.9 Hz, Ala CH_3), 2.83 and 2.90 (2H, two m, Tau α-CH_2), 3.00 and 3.16 (2H, A and B of an ABX, *J* = 5.0, 8.9, and 13.7 Hz, Phe β-CH_2) 3.47 and 3.56 (2H, two m, Tau β-CH_2), 3.76 (3H, s, $COOCH_3$), 4.40 (1H, m, Phe α-CH), 4.47 (1H, m, Ala α-CH), 5.86 (1H, d, *J* = 9.0 Hz, Phe NH), 6.51 (1 H, d, *J* = 8.0 Hz, Ala NH), 7.09 (1 H, poorly resolved t, Tau NH), 7.19–7.32 (5H, m, aromatics), 8.08 (1H, s, HCO). Anal. Calcd for $C_{16}H_{23}N_3O_6S$: C, 49.86; H, 6.01; N, 10.90. Found: C, 49.58; H, 5.96; N, 10.96.

4.6.2. Formyl-L-methionyl-tauryl-L-alanine methyl ester (5b). From Boc-L-methionyl-tauryl-L-alanine methyl ester **4b** (0.200 g, 0.45 mmol). Silica gel flash chromatography (CH_2Cl_2/MeOH 98:2) gave the pure product, which was triturated with hexane. White solid. (0.139 g, 84%). $[\alpha]_D$ −50°. Mp 98–100 °C. IR *v*: 3415, 1739, 1674 cm^{-1}. ^1H NMR δ: 1.50 (3H, d, *J* = 7.2 Hz, Ala CH_3), 1.97–2.17 (2H, m, Met β-CH_2), 2.12 (3H, s, S-CH_3), 2.58 (2H, t, *J* = 7.1 Hz, Met γ-CH_2), 3.26 (2H, m, Tau α-CH_2), 3.65, and 3.87 (2H, two m, Tau β-CH_2), 3.80 (3H, s, $COOCH_3$), 4.28 (1H, m, Ala α-CH), 4.60 (1H, m, Met α-CH), 6.11 (1H, d, *J* = 8.4 Hz, Ala NH), 6.77 (1H, d, *J* = 8.2 Hz, Met NH), 7.57 (1H, t, *J* = 5.2 Hz, Tau NH), 8.18 (1H, s, HCO). Anal. Calcd for $C_{12}H_{23}N_3O_6S_2$: C, 39.01; H, 6.27; N, 11.37. Found: C, 38.87; H, 6.05; N, 11.12.

4.6.3. Formyl-L-phenylalanyl-tauryl-L-methionine methyl ester (5c). From Boc-L-phenylalanyl-tauryl-L-methionine methyl ester **4c** (0.200 g, 0.38 mmol). Silica gel flash chromatography ($CHCl_3$/MeOH 99:1) gave the pure product, which was crystallized from EtOAc/ hexane. White solid. (0.166 g, 98%). $[\alpha]_D$ + 26°. Mp 90–92 °C. IR *v*: 3415, 1739, 1675 cm^{-1}. ^1H NMR δ: 1.89-2.15 [5H, m, Met β-CH_2 and S-CH_3 (s at 2.11)], 2.65 (2H, m, Met γ-CH_2), 3.03–3.25 (4H, m, Tau α-CH_2, and Phe β-CH_2), 3.54 and 3.75 (2H, two m, Tau β-CH_2), 3.81 (3H, s, $COOCH_3$), 4.34 (1H, m, Met α-CH), 4.65 (1H, s, Phe α-CH), 6.10 (1H, d, *J* = 9.4 Hz, Met NH), 6.50 (1H, d, *J* = 7.4 Hz, Phe NH), 7.18–7.32 (5H, m, aromatics), 7.34 (1H, poorly resolved t, Tau NH), 8.10 (1H, s, HCO). Anal. Calcd for $C_{18}H_{27}N_3O_6S_2{\cdot}H_2O$: C, 46.64; H, 6.311; N, 9.06. Found: C, 46.67; H, 6.28; N, 9.25.

4.6.4. Formyl-L-methionyl-tauryl-(*N*-methyl)-L-phenylalanine methyl ester (5d). From Boc-L-methionyl-tauryl-L-(*N*-methyl)phenylalanine methyl ester **4d** (0.160 g, 0.30 mmol). Silica gel flash chromatography ($CHCl_3$/MeOH 98:2) gave the pure product, which was crystallized from EtOAc/hexane. White solid. (0.088 g, 55%). $[\alpha]_D$ −25°. Mp 73-75 °C. IR *v*: 3417, 1740, 1673 cm^{-1}. ^1H NMR δ: 1.85-2.20 [5H, m, Met β-CH_2 and S-CH_3 (s at 2.10)], 2.45–2.70 (3H, m, Met γ-CH_2 and 1H of Tau α-CH_2), 2.78–2.85 (4H, m, 1H of Tau α-CH_2 and N-CH_3), 2.93, and 3.36 (2H, A and B of an ABX, *J* = 5.2, 10.3, and 14.3 Hz, Phe β-CH_2), 3.48 (2H, m, Tau β-CH_2), 3.79 (3H, s, $COOCH_3$), 4.63 (1H, m, Met α-CH), 4.85 (1H, m, Phe α-CH), 6.47 (1H, d, *J* = 8.3 Hz, Met NH), 6.82 (1H, t, *J* = 4.8 Hz, Tau NH), 7.23–7.35 (5H, m, aromatics), 8.18 (1H, s, HCO). Anal. Calcd for $C_{19}H_{29}N_3O_6S_2{\cdot}0.5\ H_2O$: C, 48.70; H, 6.45; N, 8.97. Found: C, 48.92; H, 6.29; N, 8.62.

4.6.5. Formyl-L-methionyl-(*N*-methyl)tauryl-L-phenylalanine methyl ester (5e). From Boc-L-methionyl-(*N*-methyl)tauryl-L-phenylalanine methyl ester **4e** (0.150 g, 0.28 mmol). Silica gel flash chromatography ($CHCl_3$/MeOH 98:2) gave the pure product as a colorless oil. (0.090 g, 70%). $[\alpha]_D$ −22°. IR *v*: 3411, 1744, 1684, 1644 cm^{-1}. ^1H NMR δ: 1.85 and 2.10 (2H, two m, Met β-CH_2), 2.14 (3H, s, S-CH_3), 2.58 (2H, m, Met γ-CH_2), 2.99–3.20 (4H, m, Tau α-CH_2 and Phe β-CH_2), 3.15 (3H, s, N-CH_3), 3.55 (2H, m, Tau β-CH_2), 3.80 (3H, s, $COOCH_3$), 4.49 (1H, m, Phe α-CH), 5.17 (1H, m, Met α-CH), 5.73 (1H, d, *J* = 9.0 Hz, Phe NH), 6.39 (1H, d, *J* = 8.0 Hz, Met NH), 7.24–7.49 (5H, m, aromatics), 8.19 (1H, s, HCO). Anal. Calcd for $C_{19}H_{29}N_3O_6S_2$: C, 49.66; H, 6.36; N, 9.14. Found: C, 49.38; H, 6.13; N, 9.02.

4.6.6. Formyl-L-methionyl-homotauryl-L-phenylalanine methyl ester (5f). From Boc-L-methionyl-homotauryl-L-phenylalanine methyl ester **4f** (0.110 g, 0.21 mmol). Silica gel flash chromatography ($CHCl_3$/MeOH 98:2) gave the pure product as a colorless oil. (0.051 g, 53%). $[\alpha]_D$ = −49°. IR *v*: 2957, 1743, 1669 cm^{-1}. ^1H NMR δ: 1.78 (2H, m, HTau β-CH_2), 1.95 and 2.08 (2H, two m, Met β-CH_2), 2.10 (3H, s, S-CH_3), 2.51 (2H, m, Met β-CH_2), 2.73 (2H, m, HTau α-CH_2), 2.90–341 (4H, three m, Phe β-CH_2 and HTau γ-CH_2), 3.78 (3H, s, $COOCH_3$), 4.37 (1H, m, Phe α-CH), 4.61 (1H, m, Met α-CH), 5.47 (1H, poorly resolved d, Phe NH), 6.65 (1H, d, *J* = 8.9 Hz, Met NH), 6.78 (1H, t, *J* = 4.3 Hz, HTau NH), 7.20–7.34 (5H, m, aromatics), 8.17 (1H, s, HCO). Anal. Calcd for $C_{19}H_{29}N_3O_6S_2$: C, 49.66; H, 6.36; N, 9.14. Found: C, 49.77; H, 6.18; N, 8.96.

4.7. Biological assays. Cell preparation

Cells were obtained from the blood of healthy subjects, and human peripheral blood neutrophils were purified by using the standard techniques of dextran (Pharmacia, Uppsala, Sweden) sedimentation, centrifugation on Ficoll–Paque (Pharmacia), and hypotonic lysis of contaminating red cells. Cells were washed twice and resuspended in Krebs–Ringer phosphate (KRPG), pH 7.4, at a final concentration of 50×10^6 cells/ml and kept at room temperature until used. Neutrophils were 98–100% viable, as determined using the Trypan blue exclusion test.

4.7.1. Random locomotion. Random locomotion was performed with 48-well microchemotaxis chamber (Bio Probe, Milan, Italy) and migration into the filter was evaluated by the leading-front method [21]. The actual control random movement is 35 ± 3 μm SE of ten separate experiments performed in duplicate.

4.7.2. Chemotaxis. Each peptide was added to the lower compartment of the chemotaxis chamber. Peptides were diluted from a stock solution (10^{-2} M in DMSO) with

KRPG containing 1 mg/ml of bovine serum albumin (BSA; Orha Behringwerke, Germany) and used at concentrations ranging from 10^{-12} to 10^{-5} M. Data were expressed in terms of chemotactic index (C.I.), which is the ratio: (migration toward test attractant − migration toward the buffer)/migration toward the buffer; the values are means of six separate experiments performed in duplicate. Standard errors are in the 0.02–0.09 C.I. range.

4.7.3. Superoxide anion (O_2^-) production. This anion was measured by the superoxide dismutase-inhibitable reduction of ferricytochrome *c* (Sigma, USA) modified for microplate-based assays. Tests were carried out in a final volume of 200 ml containing 4×10^5 neutrophils, 100 nmol cytochrome *c*, and KRPG. At zero time different amounts (10^{-10}–8×10^{-5} M) of each peptide were added and the plates were incubated into a microplate reader (Ceres 900, Bio-TeK Instruments, Inc.) with the compartment temperature set at 37 °C. Absorbance was recorded at 550 and 468 nm. The difference in absorbance at the two wavelengths was used to calculate nmol of O_2^- produced using an absorptivity for cytochrome *c* of 18.5 mM^{-1} cm^{-1}. Neutrophils were incubated with 5 μg/ml cytochalasin B (Sigma) for 5 min prior to activation by peptides. Results were expressed as net nmol of O_2^- per 1×10^6 cells per 5 min and are means of six separate experiments performed in duplicate. Standard errors are in 0.1–4 nmol O_2^- range.

4.7.4. Enzyme assay. The release of neutrophil granule enzymes was evaluated by determination of lysozyme activity, modified for microplate-based assays. Cells, 3×10^6/well, were first incubated in triplicate wells of microplates with 5 μg/ml cytochalasin B at 37 °C for 15 min and then in the presence of each peptide at a final concentration of 10^{-10}–8×10^{-5} M for a further 15 min. The plates were then centrifuged at 400*g* for 5 min and the lysozyme was quantified nephelometrically by the rate of lysis of cell wall suspension of *Micrococcus lysodeikticus*. The reaction rate was measured using a microplate reader at 465 nm. Enzyme release was expressed as a net percentage of total enzyme content released by 0.1% Triton X-100. Total enzyme activity was 85 ± 1 mg per 1×10^7 cells/min. The values are means of five separate experiments done in duplicate. Standard errors are in the range 1–6%.

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References and notes

1. Obreza, A.; Gobec, S. *Curr. Med. Chem.* **2004**, *11*, 3263.
2. Merricks, D.; Sammes, P. G.; Walker, E. R. H.; Henrick, K.; McPartlin, M. M. *J. Chem. Soc. Perkin. Trans.* **1991**, *1*, 2169.
3. Sommerfeld, T.; Seebach, D. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 553.
4. Seebach, D.; Matthews, J. L. *Chem. Commun.* **1997**, *79*, 2015.
5. Gademann, K.; Hintermann, T.; Schreiber, J. V. *Curr. Med. Chem.* **1999**, *6*, 905.
6. Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219.
7. Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893.
8. Giordano, C.; Nalli, M.; Paglialunga Paradisi, M.; Sansone, A.; Lucente, G.; Spisani, S. *Farmaco* **2004**, *59*, 953.
9. (a) Giordano, C.; Lucente, G.; Nalli, M.; Paglialunga Paradisi, M.; Varani, K.; Spisani, S. *Farmaco* **2003**, *58*, 1121; (b) Giordano, C.; Lucente, G.; Mollica, A.; Nalli, M.; Pagani Zecchini, G.; Paglialunga Paradisi, M.; Gavuzzo, E.; Mazza, F.; Spisani, S. *J. Peptide. Sci.* **2004**, *10*, 510.
10. Becker, E. L.; Freer, R. J.; Toniolo, C.; Balaram, P. In *Membrane Receptors and Cellular Regulation*; Czech, M. P., Kahn, C. R., Eds.; A.R. Liss: New York, 1985; pp 129–134.
11. Le, Y.; Zhou, Y.; Tao, H.; Wang, J. M. *Clin. Exp. All Rev.* **2004**, *4*, 155, and references therein.
12. Rathore, R. S. *Biopolymers (Peptide. Sci.)* **2005**, *80*, 651–664, and references therein.
13. Pagani Zecchini, G.; Morera, E.; Nalli, M.; Paglialunga Paradisi, M.; Lucente, G.; Spisani, S. *Farmaco* **2001**, *56*, 851.
14. (a) Freer, R. J.; Day, A. R.; Radding, J. A.; Schiffmann, E.; Aswanikumar, S.; Showell, H. J.; Becker, E. L. *Biochemistry* **1980**, *19*, 2404; (b) Freer, R. J.; Day, A. R.; Muthukumaraswamy, N.; Pinon, D.; Wu, A.; Showell, H. J.; Becker, E. L. *Biochemistry* **1982**, *21*, 257.
15. Mills, J. S.; Miettinen, H. M.; Cummings, D.; Jesaitis, A. J. *J. Biol. Chem.* **2000**, *275*, 39012, and references therein.
16. Le, Y.; Murphy, P. M.; Wang, J. M. *Trends. Immunol.* **2002**, *23*, 541, and references therein.
17. Liskamp, R. M.; Kruijtzer, J. A. W. *Mol. Divers.* **2004**, *8*, 79.
18. Wels, B.; Kruijtzer, J. A. W.; Garner, K. M.; Adan, R. A. H.; Liskamp, R. M. *J. Bioorg. Med. Chem. Lett.* **2005**, *15*, 287, and references therein.
19. Calcagni, A.; Gavuzzo, E.; Lucente, G.; Mazza, F.; Morera, E.; Paglialunga Paradisi, M.; Rossi, D. *Biopolymers* **2000**, *54*, 379.
20. Fontana, M.; Pinnen, F.; Lucente, G.; Pecci, L. *Cell Mol. Life Sci.* **2002**, *59*, 546.
21. Gennari, C.; Longari, C.; Resse, S.; Salom, B.; Mielgo, A. *Eur. J. Org. Chem.* **1998**, 945.
22. Luisi, G.; Pinnen, F. *Arch. Pharm. (Weinheim)* **1993**, *326*, 139.
23. Lajoie, G.; Kraus, J.-L. *Peptides* **1984**, *5*, 653.
24. (a) Valle, G.; Bonora, G. M.; Toniolo, C. *Gazz. Chim. Ital.* **1984**, *114*, 341; (b) Wu, Y.-D.; Wang, D.-P. *J. Am Chem. Soc.* **1998**, *120*, 13485;; (c) Möhle, K.; Günther, R.; Thormann, M.; Sewald, N. *Biopolymers* **1999**, *50*, 167.
25. Cavicchioni, G.; Breveglieri, A.; Boggian, M.; Vertuani, G.; Reali, E.; Spisani, S. *J. Peptide. Sci.* **1996**, *2*, 135.
26. Spisani, S.; Breveglieri, A.; Fabbri, E.; Vertuani, G.; Rizzati, O.; Cavicchioni, G. *Peptide. Res.* **1996**, *6*, 279.
27. Bonora, G. M.; Toniolo, C.; Freer, R. J.; Becker, E. L. *Biochem. Biophys. Acta.* **1986**, *884*, 545.
28. Miyazaki, M.; Kodama, H.; Fujita, I.; Hamasaki, Y.; Miyazaki, S.; Kondo, M. *J. Biochem.* **1995**, *117*, 489.

29. Brouwer, A. J.; Monnee, M. C. F.; Liskamp, R. M. *J. Synth.* **2000**, 1579.
30. Calcagni, A.; Gavuzzo, E.; Lucente, G.; Mazza, F.; Pinnen, F.; Pochetti, G.; Rossi, D. *Int. J. Pept. Protein Res.* **1989**, *34*, 471.
31. Guegan, R.; Diaz, J.; Cazaubon, C.; Beaumont, M.; Carlet, C.; Clement, J.; Demarne, H.; Mellet, M.; Richaud, J.-P.; Segondy, D.; Vedel, M.; Gagnol, J.-P.; Roncucci, R.; Castro, B.; Corvol, P.; Evin, G.; Roques, B. P. *J. Med. Chem.* **1986**, *29*, 1152.