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Design, synthesis, and in vitro evaluation of inhibitors of human leukocyte elastase based on a functionalized cyclic sulfamide scaffold

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Abstract—The design of novel functionalized templates capable of binding to the active site of serine proteases could potentially lead to the development of potent and highly selective non-covalent inhibitors of these enzymes. Using the elastase–turkey ovomucoid inhibitor complex and insights gained from earlier work based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold (I), a surrogate cyclosulfamide scaffold (II) was used for the first time in the design of reversible inhibitors of human leukocyte elastase. Compounds **7** and **8** were found to be micromolar reversible inhibitors of the enzyme.

1. Introduction

Mammalian proteases, as well as proteases of bacterial, viral and parasitic origin, have been implicated in a range of human diseases; consequently, these enzymes have been the focus of intense study, and as potential targets for the development of novel therapeutic agents.[1–3] The design of functionalized templates that mimic the backbone conformation of a known protein substrate (or inhibitor) of a protease that is capable of orienting recognition elements appended to it in the same vector relationship as the side chains of the amino acids that make up the substrate (or inhibitor) recognition loop offers many potential advantages, including optimal enzyme selectivity and potency.

We have recently described the structure-based design of a highly functionalized heterocyclic scaffold (I) and have demonstrated that derivatives based on (I) function as potent covalent inhibitors of serine proteases.[4–8] Scaffold (I) makes possible the exploitation of favorable binding interactions with both the S and S′ subsites and, potentially, subtle structural differences in the S and S′ subsites of closely related proteases, namely, proteases in a given class that have the *same* primary substrate specificity. Based on our findings with (I), we reasoned that functionalized surrogate scaffolds capable of mimicking (I) in terms of the spatial orientation of attached recognition elements, may lead to new classes of serine protease inhibitors and, more importantly, provide a structural framework for the rational design of inhibitors of this class of enzymes. Indeed, studies with functionalized 4-imidazolidinone derivatives have provided evidence in support of this hypothesis.[9] Further validation of this approach using functionalized cyclic sulfamide derivatives is reported herein (Fig. 1).

1.1. Chemistry

Inhibitors **7**–**8** were readily synthesized using the reaction sequence shown in Scheme 1.[10]

1.2. Biochemical studies

The inhibitory activity of compounds **7**–**8** was evaluated by determining the K_I using Dixon plots.[11] A representative experiment was carried out according to the following procedure: to a thermostatted solution of 930 μL 0.1 M HEPES buffer containing 0.5 M NaCl, pH 7.25, was added DMSO (50 μL), human leukocyte elastase (10 μL solution in 0.05 M sodium acetate buffer containing 0.5 M NaCl, pH 5.5, for a final enzyme concentration of 70 nM) and, lastly, 10 μL methoxy-succinyl-Ala-Ala-Pro-Val *p*-nitroanilide in DMSO for a final substrate concentration of 0.70 mM. The rate of hydrolysis was then determined by monitoring the

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absorbance at 410 nm for 2 min. The experiment was repeated in the presence of varying amounts of inhibitor **8** (10–50 μL) and the rates of substrate hydrolysis determined. The series of experiments described above was repeated at an additional substrate concentration (2.33×10^{-2} M). All rates were determined in triplicate. The inverse of the average velocities was plotted against the final inhibitor concentration and the K_I was determined from the intersection of the three lines (each $R^2>0.99$). A representative 1/v versus [I] plot is shown in Figure 2.

2. Results and discussion

Earlier work with the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold (I) suggested that the heterocyclic scaffold had the potential of serving as a prototype structure for the design of a series of related surrogate scaffolds with wide applicability, including the development of potent and selective non-covalent inhibitors of (chymo)trypsin-like serine proteases, the parallel synthesis of libraries of compounds, and as pharmacological probes. We reasoned that the replacement of the carbonyl group in (I) with a CH_2 group would yield a functionalized cyclic sulfamide structure (II) that could function as a *reversible* competitive inhibitor of a serine protease (Fig. 1).

To explore these ideas, we chose to conduct the biochemical studies using human leukocyte elastase (HLE), a prototypical neutrophil-derived serine protease of clinical relevance. HLE has been the focus of intense investigation because of its likely involvement in a range of diseases, including chronic obstructive pulmonary disease,[12,13] adult respiratory distress syndrome, cancer,[14,15] and others.[16,17] The recent introduction of Sivelestat (Elaspol^R), an inhibitor of HLE, for the treatment of acute lung injury associated with systemic inflammatory response syndrome, attests to the clinical potential of HLE inhibitors. Inhibitors **7** and **8** were synthesized as illustrated in Scheme 1. Both inhibitors incorporate in their structures appropriate recognition elements, specifically, a Val-Pro segment intended to interact with the S_3–S_2 subsites of the enzyme, a leucine P_1 residue that is accommodated at the primary specificity site (S_1) of the enzyme and an aromatic residue that fits into the S_2' subsite.[18] The composite effect of favorable binding interactions (Fig. 3) was anticipated to lead to the formation of a stable EI complex.

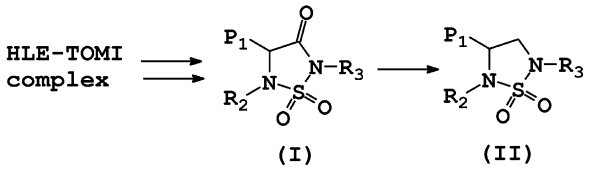
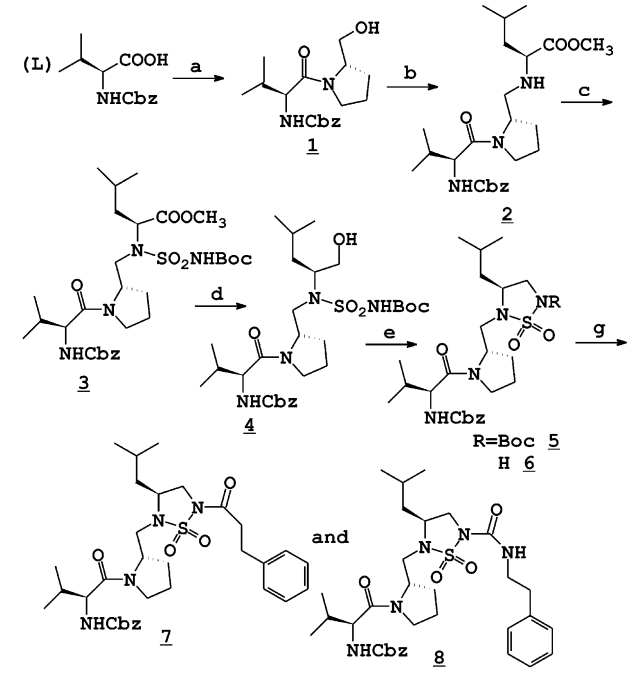


Figure 1. Evolution of the design of inhibitor (II) beginning with the human leukocyte elastase–turkey ovomucoid inhibitor (HLE–TOMI) complex.



Scheme 1. Synthesis of cyclosulfamide inhibitors **7**–**8**: (a) EDCI/HOBt/DIEA then (L) prolinol; (b) $(ClCO)_2$/DMSO/TEA then (L) Leu-OCH_3/NaBH $(OAc)_3$/HOAc; (c) $ClSO_2N{=}C{=}O$/*t*-BuOH/TEA; (d) $LiBH_4$/THF; (e) Ph_3P/DEAD/THF; (f) CF_3COOH; (g) $Ph(CH_2)_2COOH$/CDI or $Ph(CH_2)_2N{=}C{=}O$/TEA.

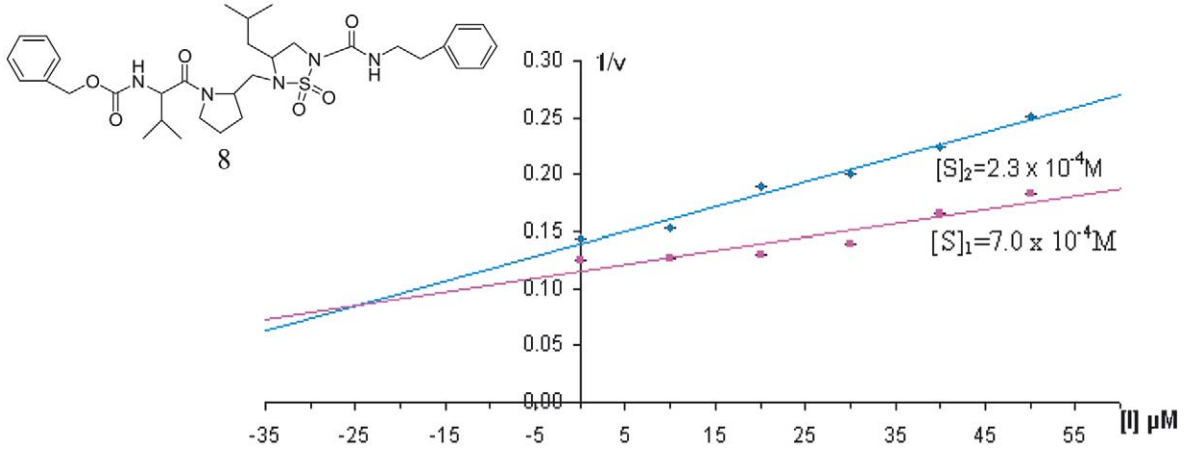


Figure 2. Dixon plot showing the inhibition of human leukocyte elastase by compound **8**.

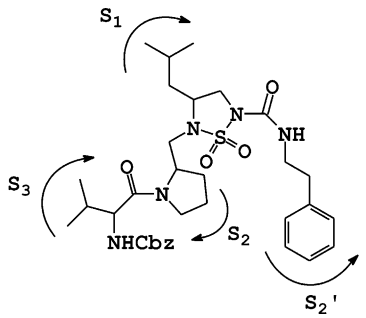


Figure 3. Main-chain and side-chain binding interactions between inhibitor **8** and the active site of HLE.

Compounds **7** and **8** were both found to be reversible competitive inhibitors of HLE (K_I 50.0 and 25.0 μM, respectively). While the potency of compounds **7** and **8** is modest, the results support the notion that the cyclic sulfamide template can be used in the design of HLE inhibitors and, possibly, related serine proteases. Furthermore, lead optimization can be attained by exploiting the three points of diversity present in (II) using focused combinatorial libraries.

In conclusion, the results of these studies provide additional support for the hypothesis that surrogate scaffolds based on (I) such as, for example, substituted 4-imidazolidinones and cyclic sulfamides (II), can be used in the design of inhibitors of serine proteases. The results of ongoing studies with a range of related surrogate templates will be reported in due course.

3. Experimental

3.1. General

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra of the synthesized compounds were recorded using either a Varian XL-300 or a Varian XL-400 NMR spectrometer. Purification of the synthesized compounds was carried out using flash chromatography with silica gel as the stationary phase. A Hewlett-Packard UV/VIS spectrophotometer was used in the enzyme assays and inhibition studies. Human leukocyte elastase was purchased from Elastin Products Company, Owensville, MO, USA. The elastase substrate, methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide, was purchased from Sigma Chemicals Co., St. Louis, MO, USA. 2 M Lithium borohydride in THF was purchased from Aldrich Chemical Co.

3.2. Synthesis of Cbz-L-Val-L-prolinol 1

A solution of Cbz-L-valine (10.7 g; 42.7 mmol), (L) prolinol (5.11 g; 49.3 mmol), diisopropylethylamine (7.10 g; 46.0 mmol) and N-hydroxybenzotriazole hydrate (16.7 g; 108.8 mmol) in DMF (80 mL) was kept in an ice-bath and treated with EDCI (9.88 g; 50.5 mmol) in one portion. The ice-bath was removed and the reaction mixture was stirred at room temperature for 11 h. Most of the DMF was removed in vacuo. Ethyl acetate (300 mL) was added and the resulting solution was washed with saturated aqueous sodium bicarbonate (300 mL). The aqueous layer was extracted with ethyl acetate (2×150 mL) and the combined organic phase was washed with 5% aqueous HCl (2×150 mL), saturated aqueous $NaHCO_3$ (2×150 mL), brine (2×150 mL), and dried over anhydrous sodium sulfate. The solvent was removed and the crude product was purified by flash chromatography (ethyl acetate/hexane) to give an oily product (11.6 g; 81% yield). ^1H NMR ($CDCl_3$): δ 0.85–1.06 (dd, 6H), 1.57 (m, 1H), 1.75–2.10 (m, 4H), 3.40–3.70 (m, 3H), 3.82 (m, 1H), 4.23 (m, 1H), 4.35 (t, 1H), 4.56 (m, 1H), 5.07 (q, 2H), 5.61 (d, 1H), 7.30–7.43 (m, 5H). Anal. calcd for $C_{18}H_{26}N_2O_4$: C, 64.65; H, 7.84; N. 8.38. Found: 64.86; H, 7.44; N, 8.19.

3.3. Synthesis of Cbz-L-Val-L-Pro-L-Leu-OCH_3 2

To a solution of 2 M oxalyl chloride in methylene chloride (48.9 mL; 97.8 mmol) kept in an acetone-dry ice bath was added dropwise a solution of dry DMSO (10.7 mL; 146.8 mmol) in dry methylene chloride (85 mL) over a period of 20 min. After stirring for 30 min, the reaction mixture was treated with a solution of compound **1** (21.8 g; 65.2 mmol) in dry methylene chloride (170 mL) dropwise over a period of 20 min. After stirring the solution for 50 min, a solution of dry TEA (20.7 g; 202.2 mmol) in methylene chloride (85 mL) was added dropwise over a period of 15 min. The acetone–dry ice bath was removed and the reaction mixture was allowed to warm up to room temperature while stirring. After 15 min, glacial acetic acid was added until the pH of the reaction mixture was about 4. (L) Leucine methyl ester hydrochloride (11.9 g; 65.2 mmol) was then added, followed by sodium (triacetoxy)borohydride (20.4 g; 91.3 mmol). The resulting solution was stirred at room temperature for 4 h. The reaction mixture was cooled in an ice-bath and the pH adjusted to 9–10 by adding 10% aqueous NaOH solution dropwise while stirring. The aqueous layer was separated and extracted with ethyl acetate (3×300 mL). The combined organic extracts were dried over anhydrous sodium sulfate and the solvent removed on the rotary evaporator, leaving a crude product which was purified by flash chromatography (silica gel/ethyl ether:hexane) to give compound **2** (24.8 g; 82% yield). ^1H NMR ($CDCl_3$): δ 0.80–1.02 (m, 12H), 1.41 (m, 2H), 1.72 (m, 1H), 1.78–2.04 (m, 5H), 2.60 (m, 2H), 3.25 (t, 1H), 3.45 (m, 1H), 3.67 (s, 3H), 4.20 (m, 1H), 4.24–4.40 (m, 1H), 5.07 (q, 2H), 5.48–5.61 (m, 1H), 7.20–7.42 (m, 5H). Anal. calcd for $C_{25}H_{39}N_3O_5$: C, 65.05; H, 8.52; N. 9.10. Found: 64.84; H, 8.80; N, 8.99.

3.4. Synthesis of 3

A solution of *N*-chlorosulfonyl isocyanate (0.13 g; 0.91 mmol) in dry methylene chloride (2 mL) was cooled in an ice-bath and a solution of *t*-butyl alcohol (0.07 g; 0.91 mmol) in dry methylene chloride (1 mL) was then added. After stirring for 15 min at 0 °C, the resulting mixture was added dropwise to a solution of compound **2** (0.42 g; 0.91 mmol) and TEA (0.10 g; 0.91 mmol) in dry methylene chloride (1 mL) kept in an ice-bath. The ice-bath was removed and the reaction mixture was

stirred for 3.5 h. The reaction mixture was diluted with methylene chloride (50 mL) and washed with water (2×15 mL) and brine (15 mL).The organic phase was separated and then dried over anhydrous sodium sulfate. Removal of the solvent gave a crude product which was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give compound **3** (0.47 g; 80% yield). Anal. calcd for $C_{30}H_{48}N_4O_9S$: C, 56.23; H, 7.55; N. 8.74. Found: 55.93; H, 7.60; N, 8.51.

3.5. Synthesis of 4

A solution of compound **3** (25.3 g; 44.8 mmol) in dry THF (70 mL) was added dropwise a solution of 2 M lithium borohydride in THF (33.6 mL, 67.20 mmol), followed by the dropwise addition of absolute ethanol (140 mL). The reaction mixture was stirred for 24 h at room temperature and then cooled in an ice-bath. A solution of 5% aq HCl was added dropwise to pH 4 with stirring. The solvent was removed to near dryness and water (150 mL) was added. The resulting mixture was extracted with ethyl acetate (3×300 mL) and the organic phase was combined and dried over anhydrous sodium sulfate. The solvent was removed and the crude product was purified by flash chromatography using silica gel and ethyl acetate/hexane as eluents to give compound **4** (16.0 g; 58% yield). ^{1}H NMR ($CDCl_3$): δ 0.81–1.05 (m, 12H), 1.35–1.54 (m, 10H), 1.54–1.64 (m, 1H), 1.75 (m, 1H), 1.82–2.10 (m, 4H), 2.21 (m, 1H), 3.20 (d, 1H), 3.33–3.60 (m, 3H), 3.60–3.95 (m, 3H), 4.05 (br s, 1H), 4.20–4.60 (m, 2H), 5.00–5.22 (m, 2H), 5.53–5.75 (dd, 1H), 7.20–7.42 (m, 5H). Anal. calcd for $C_{29}H_{48}N_4O_8S$: C, 56.84; H, 7.90; N. 9.14. Found: 56.90; H, 8.20; N, 9.06.

3.6. Synthesis of 5

A solution of compound **4** (14.8 g; 24.12 mmol) in dry THF (70 mL) was treated with triphenyl phosphine (12.82 g; 48.2 mmol) and diethyl azodicarboxylate (7.58 mL; 48.2 mmol). The reaction mixture was stirred for 3 h at room temperature. The solvent was removed on the rotovac and the crude product was purified using flash chromatography (silica gel/ethyl acetate/hexane) to give compound **5** (13.7 g; 95% yield). ^{1}H NMR ($CDCl_3$): δ 0.84–1.05 (m, 12H), 1.20–1.70 (m, 12H), 1.84–2.18 (m, 5H), 3.08 (d, 2H), 3.38 (t, 1H), 3.55 (m, 2H), 3.65 (m, 1H), 3.86 (dd, 1H), 4.20 (m, 1H), 4.30 (m, 1H), 5.18 (q, 2H), 5.40–5.55 (m, 1H), 7.24–7.42 (m, 5H). Anal. calcd for $C_{29}H_{46}N_4O_7S$: C, 56.56; H, 7.80; N. 9.42. Found: 57.38; H, 8.14; N, 9.28.

3.7. Synthesis of 6

A solution of compound **5** (13.3 g; 22.4 mmol) in dry methylene chloride (10 mL) was treated with trifluoroacetic acid (45 mL). The reaction mixture was stirred for 3 h at room temperature and the solvent was removed on a rotary evaporator to near dryness. Methylene chloride (150 mL) was added and the resulting solution was washed with 5% aq $NaHCO_3$ (2×40 mL) and brine (40 mL), and the organic phase was dried over anhydrous sodium sulfate. The solvent was removed and the crude product was purified by flash chromatography (ethyl acetate/hexane) to give compound **6** (8.81 g; 77% yield). ^{1}H NMR ($CDCl_3$) δ 0.70–1.10 (m, 12H), 1.48–1.70 (m, 2H), 1.75–2.18 (m, 5H), 2.24 (m, 1H), 2.63 (dd, 1H), 3.03–3.30 (m, 4H), 3.37 (m, 1H), 3.50–3.72 (m, 3H), 4.16–4.50 (m, 2H), 4.80–5.23 (m, 3H), 5.43–5.83 (dd, 1H), 5.95 (t, 1H), 7.23–7.40 (m, 5H). Anal. calcd for $C_{24}H_{38}N_4O_5S$: C, 58.28; H, 7.74; N. 11.33. Found: 58.40; H, 7.80; N, 11.17.

3.8. Synthesis of 7

To a solution of dihydrocinnamic acid (0.18 g; 1.20 mmol) in dry THF (2 mL) was added dropwise a solution of carbonyl diimidazole (CDI) (0.20 g; 1.20 mmol) in dry THF (3 mL). The resulting solution was stirred for 30 min at room temperature and compound **6** (0.59 g; 1.20 mmol) was added in one portion. After stirring the resulting solution for 15 min at room temperature, a solution of 1,8-diazabicyclo [5.4.0]-undec-7-ene (DBU) (0.19 g; 1.20 mmol) in dry THF (5 mL) was added dropwise. The reaction solution was stirred for 2 h at room temperature. The solvent was removed and ethyl acetate (50 mL) was added. The resulting solution was washed with 5% aqueous HCl (15 mL), 5% aq $NaHCO_3$ (15 mL) and brine (15 mL). The organic phase was dried over anhydrous sodium sulfate. Removal of the solvent yielded a crude product, which was purified by flash chromatography (ethyl acetate/hexane) to give compound **7** (0.54 g; 72% yield). Anal. calcd for $C_{33}H_{46}N_4O_6S$: C, 63.26; H, 7.40; N; 8.95. Found: 63.15; H, 7.25; N, 8.81.

3.9. Synthesis of 8

A solution of compound **6** (0.43 g; 0.87 mmol) and TEA (0.13 g; 1.22 mmol) in dry methylene chloride (3 mL) was treated with 2-phenethyl isocyanate (0.24 g; 1.57 mmol). The reaction mixture was refluxed for 2.5 h with stirring. Following removal of most of the solvent, 5% aqueous HCl (25 mL) was added, and the resulting mixture was then extracted with ethyl acetate (2×75 mL). The organic extracts were combined and dried over anhydrous sodium sulfate. The solvent was removed and the crude product was purified by flash chromatography (ethyl acetate/hexane) to give compound **8** (0.55 g; 98% yield). Anal. calcd for $C_{33}H_{47}N_5O_6S$: C, 61.75; H, 7.38; N; 10.91. Found: 61.48; H, 7.73; N, 10.72.

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References and notes

1. Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.
2. Maly, D. J.; Huang, L.; Ellman, J. A. *ChemBiochem.* **2002**, *3*, 16.

3. Zhong, J.; Groutas, W. C. *Curr. Top. Med. Chem.* In press.
4. Groutas, W. C.; Epp, J. B.; Kuang, R.; Ruan, S.; Chong, L. S.; Venkataraman, R.; Tu, J.; He, S.; Yu, Q.; Fu; Li, Y. H.; Truong, T. M.; Vu, N. T. *Arch. Biochem. Biophys.* **2001**, *385*, 161.
5. Groutas, W. C.; He, S.; Kuang, R.; Ruan, S.; Tu, J.; Chan, H.-K. *Bioorg. Med. Chem.* **2002**, *9*, 1543.
6. Kuang, R.; Epp, J. B.; Ruan, S.; Chong, L. S.; Venkataraman, R.; Tu, J.; He, S.; Truong, T. M.; Groutas, W. C. *Bioorg. Med. Chem.* **2000**, *8*, 1005.
7. Kuang, R.; Epp, J. B.; Ruan, S.; Yu, H.; Huang, P.; He, S.; Tu, J.; Schechter, N. M.; Turbov, J.; Froelich, C. J.; Groutas, W. C. *J. Am. Chem. Soc.* **1999**, *121*, 8128.
8. Groutas, W. C.; Kuang, R.; Venkataraman, R.; Epp, J. B.; Ruan, S.; Prakash, O. *Biochemistry* **1997**, *36*, 4739.
9. Wei, L.; Gan, X.; Zhong, J.; Alliston, K.; Groutas, W. C. *Bioorg. Med. Chem.* **2003**, *11*, 5149.
10. Regainia, Z.; Abdaoui, M.; Auf, N.-E.; Dewynter, G.; Montero, J.-L. *Tetrahedron* **2000**, *56*, 381.
11. Dixon, M. *Biochem. J.* **1953**, *55*, 170.
12. Ohbayashi, H. *Expert Opin. Investig. Drugs* **2002**, *11*, 965.
13. Zeiher, B. G.; Matsuoka, S.; Kawabata, K.; Repine, J. E. *Crit. Care Med.* **2002**, *30*, S281.
14. Shamanian, P.; Pocock, B. J. Z.; Schwartz, J. D.; Monea, S.; Chuang, N.; Whiting, D.; Marcus, S. G.; Galloway, A. C.; Mignatti, P. *Surgery* **2000**, *126*, 142.
15. Fockens, J. A.; Ries, C.; Look, M. P.; Gippner-Steppert, C.; Klijn, J. G.; Jochum, M. *Cancer Res.* **2003**, *63*, 337.
16. Metz, W. A.; Peet, N. P. *Exp. Opin. Ther. Pat.* **1999**, *9*, 851.
17. Anderson, G. P.; Shinagawa, K. *Curr. Opin. Anti-inflamm. Immunom. Invest. Drugs* **1999**, *1*, 29.
18. Schechter, I.; Berger, A. *J. Biol. Chem.* **1962**, *12*, 2940.