Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5997–6000
Bioorganic & Medicinal Chemistry Letters

Isoxazole-3-hydroxamic acid derivatives as peptide deformylase inhibitors and potential antibacterial agents

Patrizia Calí,[a,*] Lars Nærum,[a] Seema Mukhija[b] and Anders Hjelmencrantz[a]

[a]*Combio A/S, Vesterbrogade 188, DK-1800 Frederiksberg C, Denmark*
[b]*Arpida Ltd, Dammstrasse 36, 4142 Münchenstein, Switzerland*

Received 2 June 2004; revised 29 August 2004; accepted 30 September 2004
Available online 18 October 2004

Abstract—A series of isoxazole-3-hydroxamic acid derivatives has been identified as a new class of small, nonpeptidic inhibitors of peptide deformylase (PDF). The synthesis, enzyme inhibition and preliminary investigation of the binding mode of this potential antibacterial compounds are reported.

In recent years, there has been a growing interest in developing bacterial peptide deformylase (PDF) inhibitors as novel antibiotics. PDF is an iron-containing metalloenzyme, which catalyses the removal of the *N*-formyl group from the terminal methionine residue of nascent proteins.[1] PDF is essential for both Gram-positive and Gram-negative bacteria, since deformylation is a necessary step to complete protein biosynthesis and maturation. Due to its wide distribution in bacteria and its absence in mammalian cells, PDF represents an attractive target for the discovery of broad spectrum antibacterial drugs.[2]

Many PDF inhibitors have been reported in recent years.[3,4] Most of the compounds with sufficient potency and antibacterial activity share a common structure, as exemplified in Figure 1. Two structural features are constantly recurrent and seem to account for most of the binding energy; a metal chelating group X, most commonly a hydroxamate or *N*-formylhydroxylamine, and a *n*-alkyl (usually *n*-butyl) residue at P1′ mimicking the methionine side chain and fitting into the deep S1′ hydrophobic pocket in the PDF active site. Examples are the natural occurring antibiotic actinonin (**1**)[5] and the reverse hydroxamate from British Biotech BB-83698 (**2**),[6] currently in phase I clinical trials (Fig. 1). However, since most of the known inhibitors still have significant peptide characteristics, there are some concerns about their selectivity and in vivo metabolic stability. Here we wish to report the synthesis and preliminary in vitro evaluation of a new series of nonpeptidic PDF inhibitors having an isoxazole-3-hydroxamic acid as central core (**3**). The binding mode of this class of inhibitors is investigated by comparison with the crystal structure of the actinonin–PDF complex.

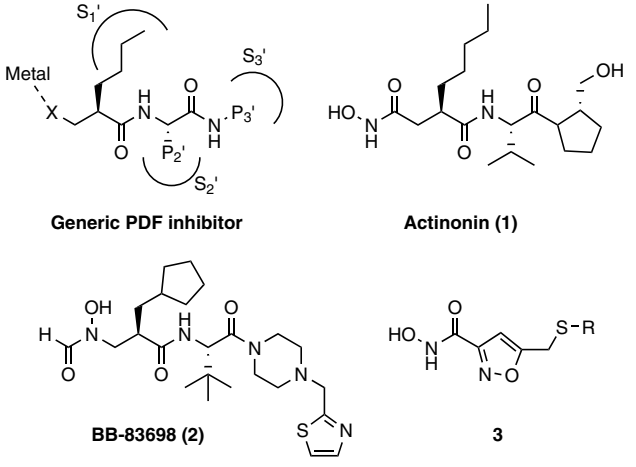
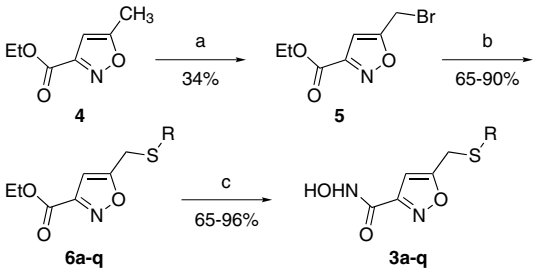


Figure 1. Structure of a generic PDF inhibitor compared to actinonin (**1**), to BB-83698 (**2**) and to the new series of isoxazole-3-hydroxamic acid derivatives (**3**).

The general route for the synthesis of the isoxazole-3-hydroxamic acids (**3**) is outlined in Scheme 1. Radical bromination of ethyl-5-methylisoxazole-3-carboxylate

Keywords: Peptide deformylase; Inhibitors; Antibacterial.
* Corresponding author. Tel.: +45 70222365; fax: +45 70222367; e-mail: pca@combio.biz

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.09.087

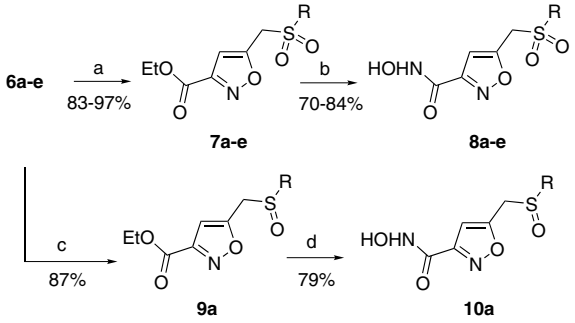


Scheme 1. Reagents and conditions: (a) NBS, BPO, CCl_4, 95 °C, 4 h, 34%; (b) RSH, K_2CO_3, DMF, 60 °C, 1 h; (c) NH_2OH, KOH, MeOH, rt, 2–24 h.

(**4**) was obtained by refluxing with *N*-bromosuccinimide and catalytic amounts (10%) of benzoylperoxide as radical initiator.[7] Selective bromination of the benzylic position over the isoxazole ring could be achieved by using equimolar amount of NBS. The intermediate benzylic bromide **5** was alkylated with an array of aromatic, heteroaromatic and benzylic thiols, in the presence of K_2CO_3. All reactions were completed after 1 h affording the desired thioethers **6a**–**q** in 65–96% yield after purification by column chromatography. The reaction of the ethyl esters **6a**–**q** with a methanolic solution of hydroxylamine hydrochloride and KOH yielded the desired hydroxamic acids **3a**–**q**.[8] All final products were purified by preparative HPLC and characterised by ^{1}H, ^{13}C NMR and LCMS analysis.

A few oxidised analogues (**8a**–**e** and **10a**) were synthesised as represented in Scheme 2. The reaction of the thioethers **6a**–**e** with *m*-chloroperbenzoic acid yielded the sulfonyl derivatives **7a**–**e**.[9] When intermediate **6a** was reacted with $NaBO_3$ at rt for 1 h, the mono-oxidised products **9a** was selectively obtained. The synthesis of the final hydroxamic acids **8a**–**e** and **10a** was performed as previously described in a methanolic solution of hydroxylamine hydrochloride and KOH.

The final hydroxamic acids were evaluated for their in vitro inhibitory activity on *Escherichia coli* and *Staphylococcus aureus* PDF enzymes.[10] The activities of the compounds compared to actinonin **1** are summarised in Table 1. Since the bidentate, metal-chelating hydroxamic acid moiety was previously shown to be



Scheme 2. Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, rt, 1–3 h; (b) NH_2OH, KOH, MeOH, rt, 2–24 h; (c) $NaBO_3 4H_2O$, AcOH, rt; 1 h; (d) NH_2OH, KOH, MeOH, rt, 2 h.

Table 1. PDF enzyme inhibitory activity of isoxazole-3-hydroxamic acids **3a**–**q**, **8a**–**e** and **10a** compared to actinonin **1**

Compds	R	*E. coli* IC_{50}, (μM)[a]	*S. aureus* IC_{50} (μM)[a]
1		0.0007	0.002
3a	4-(CH_3)Ph	20.0	4.0
3b	3-(Cl)Ph	9.8	2.3
3c	4-(Cl)Ph	14.5	1.3
3d	3-(OCH_3)Ph	6.5	2.2
3e	2-(*i*-Pr)Ph	14.0	7.6
3f	4-(OCH_3)Ph	31.3	24.0
3g	2,4,6-(CH_3)Ph	29.5	37.7
3h	2-(Cl)Ph	62.5	3.8
3i	3,4-(Cl)Ph	4.4	2.0
3j	2-(Br)Ph	60.0	4.9
3k	3-(Br)Ph	7.0	2.0
3l	4-(F)Ph	20.7	1.5
3m	2-(CF_3)Ph	25.3	9.3
3n	4-(CF_3)Ph	22.0	8.7
3o	4-($NHCOCH_3$)Ph	20.3	42.0
3p	Benzothiophen-2-ylmethyl	3.4	12.7
3q	3-(CF_3)-benzyl	7.6	2.2
8a	4-(CH_3)Ph	11.5	1.4
8b	3-(Cl)Ph	6.8	1.7
8c	4-(Cl)Ph	7.5	0.8
8d	3-(OCH_3)Ph	26.3	6.5
8e	2-(*i*-Pr)Ph	10.8	3.4
10a	4-(CH_3)Ph	12.0	0.9

[a] Values are means of three experiments.

critical for good inhibitory activity,[11] this functionality was conserved in all derivatives synthesised and tested.[12] In spite of the high similarity of the binding sites generally reported,[13] significant differences in the inhibitory activity were observed with *S. aureus* and *E. coli* PDF enzymes, the former being generally more sensitive to changes in the substitution pattern of the inhibitors.

In order to investigate the binding mode of our inhibitors and rationalise the results, compound **3b** was minimised and positioned into the crystal structure of the PDF–actinonin complex (PDB access code, 1G2A).[4f] The result of our molecular modelling study is illustrated in Figure 2.[14] The hydroxamic acid moiety of **3b** can be positioned favourably for chelating the Ni atom in the active site, in a similar fashion to actinonin. The isoxazole ring orientation allowed for a favourable H-bond between the oxygen and the backbone NH of Ile44, similarly to the P1′ carbonyl in actinonin. The distances between the actinonin-carbonyl and isoxazole-oxygen with the NH of Ile44 are 1.8 and 2.2 Å, respectively. The best fit was obtained when **3b** assumed a bent conformation, positioning the thioaromatic residue and the *meta*-chloro substituent into the hydrophobic S1′ pocket. As the *n*-pentyl group in the actinonin–PDF complex, the thioaromatic group is engaged in extensive hydrophobic interactions in the S1′ pocket with enzyme side chains. A similar binding mode was also observed with the potent PDF inhibitors by Hoffmann-La Roche (magenta in Figure 2), 2,2-dioxo-1,4-dihydrobenzo(1,2,6)thiadiazinyl hydroxamic acid, where small lipophilic groups (F, Cl, Br) were found to ideally fit into the S1′ pocket and increase the activity.[4b] Since a

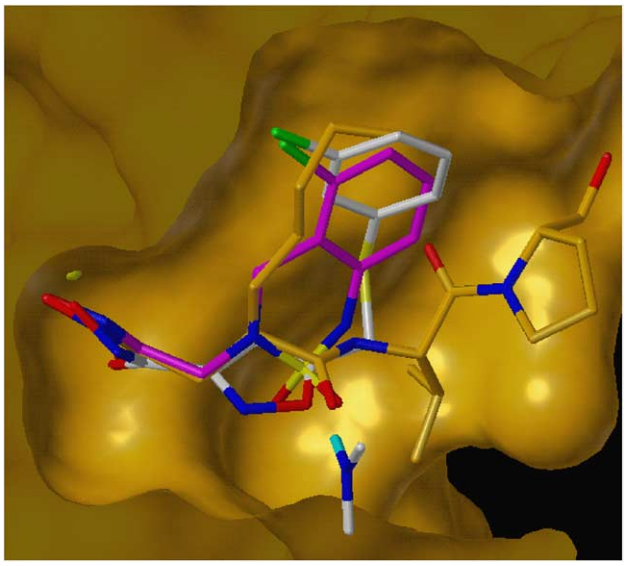


Figure 2. Molecular model predicting the binding mode of inhibitor **3b** in comparison with the actinonin–PDF complex. Actinonin is shown in gold, inhibitor **3b** is in white and the Hoffmann-La Roche inhibitor[4b] is in magenta. The surface of the enzyme (S1′ pocket) is represented in gold and the Ni^{2+} ion is represented as a yellow ball. The backbone NH of Ile44 is also highlighted.

tight binding in the S1′ pocket has proven crucial in order to obtain potent inhibitors, it can be concluded that the nature and the position of the thioaromatic substituents will play a central role in determining the affinity. As suggested from the modelling study, neither the P2′ nor the P3′ regions are addressed by compound **3b**. This could account for the much lower activity observed in the isoxazole derivatives series compared to the reference actinonin **1**.

From the modelling study we expected that small lipophilic groups (Cl, Br, CF_3) in the *meta*-position will be filling the methionine pocket optimally. In accordance with our expectations, *meta*-substitution (e.g., **3b**, **3d**, **3i**, **3k**) appeared generally favoured over *ortho*- and *para*- substitution, both with *E. coli* and *S. aureus* PDF enzymes. Derivatives **3i**, with a 3,4-dichloro substituent, was one of the most potent inhibitor of *E. coli* PDF enzyme with an IC_{50} value of 4.4 μM, and 2 times more active with *S. aureus* PDF. *ortho*-Substitution with small lipophilic groups (Cl, Br, CF_3) and alkyl chains (*i*-propyl) led to significantly less active inhibitors of *E. coli* enzyme (e.g., **3e**, **3h**, **3j**, **3m**), though the loss of activity appeared less pronounced in the case of *S. aureus* PDF. *para*-Substitution (**3a**, **3c**, **3f**, **3l**, **3n**, **3o**) generally resulted in poor inhibition of *E. coli* PDF. More dramatic is the effect of *para*-substitution with the *S. aureus* PDF enzyme, particularly with bulky and polar substituents. Activity comparable to the corresponding *meta*-substituted series, was observed with *S. aureus* PDF enzyme in the case of small lipophilic substituents, like fluorine (**3l**) or chlorine (**3c**), suggesting that these groups can still be accommodated at the end of the S1′ pocket without significant distortion from the optimal interaction. Higher IC_{50} values were observed with more bulky substituents like methyl (**3a**), methoxy (**3f**), *N*-acetyl groups (**3o**). Since the methionine pocket has proven to be very sensitive to the length of the hydrophobic chain, it can be speculated that these derivatives bind the PDF enzyme with an extended conformation, locating the thioether side chain away from the S1′ pocket. Oxidation of the thioether of derivatives **3a**–**e** to sulfoxide (**10a**) and sulfone (**8a**–**e**) only slightly improved the potency of the compounds, resulting in IC_{50} values 1.5–3 times lower than the corresponding un-oxidised compounds. Since for derivatives **3a**–**e** the sulfone oxygen is placed in close proximity to the P2′ carbonyl of actinonin, a similar interaction with the backbone NH of Gly89 could be envisaged and account for the observed difference in activity.

In addition, the whole-cell activity was assessed for the most active compounds in the series (Table 2).[15] None of the inhibitors tested showed any antibacterial activity against wild type or resistant strains of both *E. coli* and *S. aureus*, as observed for actinonin **1**. However, while actinonin displayed a strong antibacterial activity in mutant of *E. coli* EC-DC2[16] and EC-AH547, respectively, a hypersensitive and a strain with reduced activity of efflux pumps, the isoxazole derivatives showed only poor MIC values.

These results suggest that the poor antibacterial activity in Gram negatives derives from a combination of poor penetration of the bacterial cell wall and recognition

Table 2. In vitro antibacterial activity of selected isoxazole-3-hydroxamic acids

Compds	R	MIC (μg/mL)[a]				
		SA-ATCC-25923[b]	*SA*-101[b]	*EC*-ATCC-25922[c]	*EC*-DC2[c]	*EC*-AH547[d]
1		64	128	128	0.5	0.5
3b	3-(Cl)Ph	>128	>128	>128	32	64
3c	4-(Cl)Ph	>128	>128	128	32	64
3d	3-(OCH_3)Ph	>128	>128	>128	128	128
3i	3,4-(Cl)Ph	128	128	>128	32	16
3k	3-(Br)Ph	128	>128	>128	64	64
3p	Benzothiophen-2-ylmethyl	128	128	128	32	64

[a] MIC = minimum inhibitory concentration.[15]
[b] *SA*-ATCC-25923 and *SA*-101 are, respectively, the wild type and a multi resistant strain of *S. aureus*.
[c] *EC*-ATCC-25922 and *EC*-DC2 are, respectively, the wild type and a hyperpermeable strain of *E. coli*.
[d] *EC*-AH547 is a tolC mutant strain of *E. coli* with reduced activity of efflux pumps.

by an efficient efflux system of the cell, similarly to compound **1**. However, in the case of the isoxazole-3-hydroxamic acids the lack of antibacterial activity might also be related to the only moderate inhibitory activity of PDF enzyme. In fact, more potent inhibitors are generally required in order to observe significant antibacterial activity.

In conclusion, we have identified a new class of nonpeptidic PDF inhibitors. According to our initial molecular model, three key interactions occur between the inhibitors and the enzyme binding site: bidentate coordination to the metal centre, H-bond with Ile44 and hydrophobic interaction at P1′ position. The compounds studied display inhibitory activity on the enzymes from *E. coli* and *S. aureus*, though they exhibit weak antibacterial activity only in hypersensitive or efflux-defective Gram-negative strains. As suggested by the molecular model, neither the P2′ nor the P3′ regions seem addressed by the studied isoxazole derivatives. Therefore, the introduction of interactions in the P2′ and P3′ regions might improve both potency and antibacterial activity.

Acknowledgements

The authors thank all the colleagues who have contributed to the project and Dr. Peter Schneider for helpful advice.

References and notes

1. (a) Adams, J. M. *J. Mol. Biol.* **1968**, *5*, 571–589; (b) Adams, J. M.; Capecchi, M. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 147–155.
2. (a) Yuan, Z.; Trias, J.; White, R. J. *Drug Discovery Today* **2001**, *6*, 954–961; (b) Clemens, J. M.; Ayscough, A. P.; Keavey, K.; East, S. P. *Curr. Med. Chem. Anti-infective Agents* **2002**, *1*, 239–249; (c) Pei, D. *Emerging Ther. Targets* **2001**, *5*, 23–40.
3. For examples of thiol inhibitors, see: (a) Meinnel, T.; Patiny, L.; Ragusa, S.; Blanquet, S. *Biochemistry* **1999**, *38*, 4287–4295; (b) Huntington, K. M.; Yi, T.; Wei, Y.; Pei, D. *Biochemistry* **2000**, *39*, 4543–4551; (c) Wei, Y.; Yi, T. Y.; Huntingtom, K. M.; Chaudhury, C.; Pei, D. *J. Comb. Chem.* **2000**, *2*, 650–657.
4. For examples of hydroxamate and reverse hydroxamate inhibitors, see: (a) Apfel, C.; Banner, D. W.; Bur, D.; Dietz, M.; Hirata, T.; Hubschwerlen, C.; Locher, H.; Page, M. G. P.; Pirson, W.; Rossé, G.; Specklin, J. L. *J. Med. Chem.* **2000**, *43*, 2324–2331; (b) Apfel, C.; Banner, D. W.; Bur, D.; Dietz, M.; Hubschwerlen, C.; Locher, H.; Marlin, F.; Masciadri, R.; Pirson, W.; Stalder, H. *J. Med. Chem.* **2001**, *44*, 1847–1852; (c) Thorarensen, A.; Douglas, M. R., Jr.; Rohrer, D. C.; Vosters, A. F.; Yem, A. W.; Marshall, V. D.; Lynn, J. C.; Bohanon, M. J.; Tomich, P. K.; Zurenko, G. E.; Sweeney, M. T.; Jensen, R. M.; Nielsen, J. W.; Seest, E. P.; Dolak, L. A. *Bioorg. Med. Chem. Lett.* **2002**, *11*, 1355–1358; (d) Hackbart, C. J.; Chen, D. Z.; Lewis, J. G.; Clark, K.; Mangold, J. B.; Cramer, J. A.; Margolis, P. S.; Wang, W.; Koehn, J.; Wu, C.; Lopez, S.; Withers, G., III; Gu, H.; Dunn, E.; Kulathila, R.; Pan, S. H.; Porter, W. L.; Jacobs, J.; Trias, J.; Patel, D. V.; Weidmann, B.; White, R. J.; Yuan, Z. *Antimicrob. Agents Chemother.* **2002**, *46*, 2752–2764; (e) Takayama, W.; Shirasaki, Y.; Sakai, Y.; Nakajima, E.; Fujita, S.; Sakamoto-Mizukani, K.; Inoue, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3273–3276; (f) Clemens, J. M.; Beckett, R. P.; Brown, A.; Catlin, G.; Lobell, M.; Palan, S.; Thomas, W.; Whittaker, M.; Wood, S.; Salama, S.; Baker, P. J.; Rodgers, H. F.; Barynin, V.; Rice, D. W.; Hunter, M. G. *Antimicrob. Agents Chemother.* **2001**, *45*, 563–570; (g) East, S. P.; Beckett, R. P.; Brookings, D. C.; Clemens, J. M.; Doel, S.; Keavey, K.; Pain, G.; Smith, H. K.; Thomas, W.; Thompson, A. J.; Todd, R. S.; Whittaker, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 59–62; (h) Hu, X.; Nguyen, K. T.; Verlinde, C. L. M. J.; Hol, W. G. J.; Pei, D. *J. Med. Chem.* **2003**, *46*, 3771–3774.
5. Chen, D. Z.; Patel, D. V.; Hackbarth, C. J.; Wang, W.; Dreyer, J.; Young, D. C.; Margolis, P. S.; Wu, C.; Ni, Z. J.; Trias, J.; White, R. J.; Yuan, Z. *Biochemistry* **2000**, *39*, 1256–1262.
6. (a) Lofland, D.; Difuntorum, S.; Waller, A.; Clements, J. M.; Weaver, M. K.; Karlowsky, J. A.; Johnson, K. *Antimicrob. Chemother.* **2004**, *53*, 664–668; (b) Azoulay-Dupuis, E.; Mohler, J.; Bédos, J. P. *Antimicrob. Agents Chemother.* **2004**, *48*, 80–85.
7. Tashiro, M.; Yamato, T. *J. Org. Chem.* **1985**, *50*, 2939–2942.
8. Hauser, C. R.; Renfrow, W. B., Jr. *Org. Synth.* **1939**, *19*, 15–17.
9. Guzmán, A.; Romero, M.; Talamás, F. X. *J. Org. Chem.* **1996**, *61*, 2470–2483.
10. PDF inhibition assay, optimised to microtiter plate format, was performed as previously described by Groche, D.; Becker, A.; Schlichting, I.; Kabsch, W.; Schultz, S.; Wagner, A. F. *Biochem. Biophys. Res. Commun.* **1998**, *246*, 342–346.
11. Smith, H. K.; Beckett, R. P.; Clemens, J. M.; Doel, S.; East, S. P.; Launchbury, S. B.; Pratt, L. M.; Spavold, Z. M.; Thomas, W.; Todd, R. S.; Whittaker, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3595–3599.
12. The corresponding isoxazole-3-carboxylic acids of compounds **3a**–**q** were also synthesised and resulted in no inhibitory activity with PDF enzyme (data not reported).
13. For X-ray of PDF enzymes and comparison of binding sites in different bacterial strains, see: (a) Guilloteau, J. P.; Mathieu, M.; Giglione, C.; Blanc, V.; Dupuy, A.; Chevrier, M.; Gil, P.; Famechon, A.; Meinnel, T.; Mikol, V. *J. Mol. Biol.* **2002**, *320*, 951–962; (b) Kreusch, A.; Spraggon, G.; Lee, C. C.; Klock, H.; McMullan, D.; Ng, K.; Shin, T.; Vincent, J.; Warner, I.; Ericson, C.; Lesley, S. A. *J. Mol. Biol.* **2003**, *330*, 309–321.
14. Modelling study was performed using a combined FlexX and Maximin approach within Sybyl 6.8, Tripos Inc., USA.
15. Antibacterial activities were determined in liquid culture according to NCCLS standard conditions: Methods for Dilution Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Fifth Edition, **2000** NCCLS M7-A5, Vol. 20, No. 2.
16. Richmond, M. H.; Clark, D. C.; Wotton, S. *Antimicrob. Agents Chemother.* **1976**, *10*, 215–218.