Bioorganic & Medicinal Chemistry Letters 20 (2010) 1225–1228

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel small molecule bradykinin B_1 receptor antagonists. Part 1: Benzamides and semicarbazides

Marco Schaudt, Elsa Locardi, Gunther Zischinsky, Roland Stragies, Jochen R. Pfeifer, Christoph Gibson, Dirk Scharn, Uwe Richter, Holger Kalkhof, Klaus Dinkel, Karsten Schnatbaum *

Jerini AG, Invalidenstraße 130, 10115 Berlin, Germany

ARTICLE INFO

Article history:
Received 22 October 2009
Revised 23 November 2009
Accepted 24 November 2009
Available online 2 December 2009

Keywords:
Bradykinin B_1
Antagonist
Oral bioavailability
Pharmacokinetic profile

ABSTRACT

The synthesis and SAR of two series of bradykinin B_1 receptor antagonists is described. The benzamide moiety proved to be a suitable replacement for the aryl ester functionality of biaryl based antagonists. In addition, it was found that semicarbazides can effectively replace cyclopropyl amino acids. The compounds with the best overall profile were biaryl semicarbazides which display high antagonistic activity, low Caco-2 efflux and high oral bioavailability in the rat.

Kinin peptides are released from kininogen precursors by the action of plasma and tissue kallikreins in response to traumatic injury and infection.[1] The biological actions of kinins are mediated by two major G-protein coupled receptors designated as bradykinin B_1 and bradykinin B_2 receptor. The B_2 receptor is constitutively expressed under physiological conditions in a variety of cells while the B_1 receptor is induced under pathological conditions such as tissue damage or inflammation in several cell types including endothelial, smooth muscle cells, blood cells, and neurons.[2,3] This difference in expression makes the B_1 receptor a particularly attractive drug target. Activation of the B_1 receptor produces a range of pro-inflammatory effects including edema, pain, and promotion of blood-borne leukocyte trafficking.[4,5]

Recently, a number of publications and patent applications described the identification and optimization of biaryl-based bradykinin B_1 receptor antagonists such as **1** (MK-686, Fig. 1).[6,7] It was reported that **1** revealed certain metabolic liabilities related to the methyl ester moiety[8] and showed low aqueous solubility.[9] To resolve these issues, several studies were undertaken in which the biaryl region, as well as the N-terminus of the amino acid, were modified.[8–11] Here we report an alternative strategy to replace the biaryl unit and the cyclopropyl amino acid moiety (Fig. 1). Using a benzamide moiety instead of the aryl ester resulted in a novel class of B_1 receptor antagonists (**2a–g**). Additionally, semicarbazides are shown to serve as suitable replacement for the cyclopropyl amino acid moiety. Highly active compounds were thus obtained by combination of semicarbazides with benzamides (**3a–f**) or biaryls (**4a–k**).

The general synthesis of target compounds **2a–g** and **3a–f** is illustrated in Scheme 1. Ester **6** was prepared from **5**[7,12] by cyanation of the aryl bromide, hydrolysis and ester formation. The introduction of the semicarbazide group was achieved by coupling Boc-protected alkyl hydrazines[13] with phosgene followed by Boc

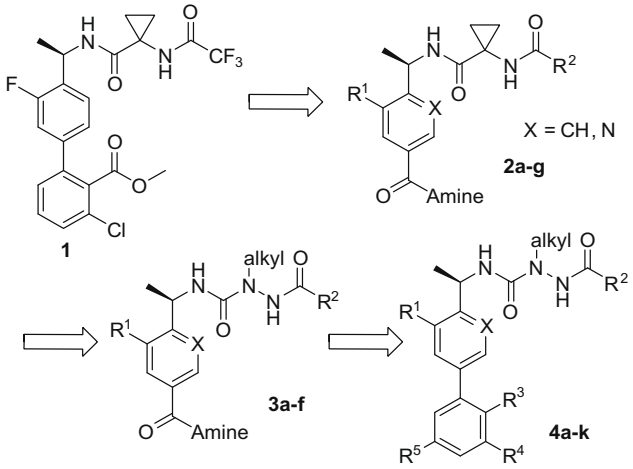
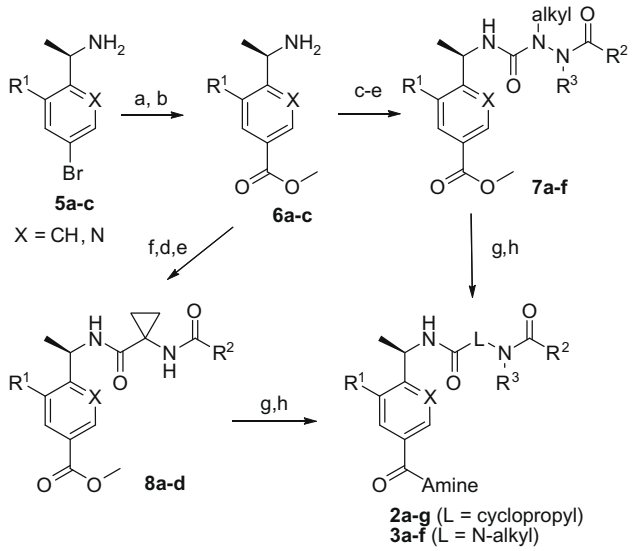


Figure 1. Design of new bradykinin B_1 receptor antagonists.

* Corresponding author. Correspondence should be addressed to: Pericles Calias, Shire HGT, 125 Spring Street, Lexington, MA 02421, USA. Tel.: +1 781 482 0701; fax: +1 781 482 2961. E-mail: pcalias@shire.com.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.11.119



Scheme 1. Reagents and conditions: (a) (i) AcCl, TEA, Et_2O; (ii) CuCN, DMF, 160 °C; (b) (i) 6 N HCl, 110 °C; (ii) MeOH, cat. HCl, 70 °C; (c) $COCl_2$, *N*′-alkyl-hydrazine-carboxylic acid *tert*-butyl ester, DIPEA, THF; (d) HCl/Methanol; (e) R^2CO_2H, HATU, DIPEA, DMF; (f) *tert*-butoxycarbonyl-aminocyclopropane carboxylic acid, HATU, DIPEA, DMF; (g) LiOH, dioxane/H_2O; (h) amine, HATU, DIPEA, DMF.

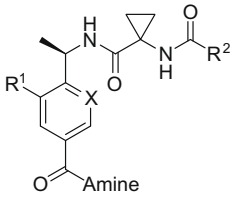
deprotection. Coupling reactions with carboxylic acid derivatives formed precursor **7**. Applying a cyclopropyl amino acid derivate in this synthesis pathway yielded precursor **8**. The final compounds **2a–g** and **3a–f** were obtained by hydrolysis of the methyl ester and HATU coupling reactions with the corresponding amines.

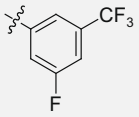
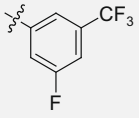
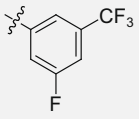
After surveying a variety of primary and secondary amines in combination with different R^2s, a focused series of benzamides was prepared (Table 1). The in vitro functional activity of the compounds at the B_1 receptor was assessed in a DAKD-mediated calcium mobilization (CAM) assay using IL-1 beta pretreated human lung fibroblasts IMR-90. The SAR of the pyrimidyl benzamides **2a** (IC_{50} = >10,000 nM) and **2b** (IC_{50} = 3100 nM) demonstrates that the secondary amine is advantageous to provide B_1 receptor antagonist activity. Increasing steric bulk from diethyl (**2b**, IC_{50} = 3100 nM) to diisopropyl (**2c**, IC_{50} = 500 nM) led to a sixfold increase in activity. Encouraged by this result, we synthesized the 2,5-dimethylpyrrolidyl amide **2d** (IC_{50} = 125 nM) which showed a fivefold increase in potency compared to the diisopropyl amide **2c**. Furthermore, switching of R^2 from the polar pyrimidine moiety (**2d**) to the bulky hydrophobic 3-fluoro-5-(trifluoromethyl)benzoic acid (**2e**) resulted in a drastic 25-fold increase of activity (**2e**, IC_{50} = 5 nM). Employing the enantiomerically pure 2,5-dimethylpyrrolidyl and fluoro substitution at R^1 led to **2f** (IC_{50} = 1.3 nM). Interestingly, and in contrast to biphenyl based B_1 receptor antagonists,[6] modification of the phenyl core to a pyridyl was not tolerated by the B_1 receptor (**2f**, IC_{50} = 1.3 nM vs **2g**, IC_{50} = 28 nM).

Compound **2f** exhibited a high aqueous solubility of 42 μM as well as a reasonable metabolic stability[14] (45% remaining after 1 h in rat microsomes) and a reasonable pharmacokinetic profile in male Wistar rat (F = 43%, $t_{1/2}$ = 39 min, CL = 34 mL/min/kg). However, the human microsomal stability of **2f** proved to be low (9% remaining after 1 h in human microsomes). Consequently, **2f** was not considered for further characterization.

At this stage we decided to focus on the exploration of the amino acid moiety. As it is well-known that aza amino acids can serve as suitable replacement for amino acids[15] we hypothesized that semicarbazides might provide viable analogues. Therefore the compounds **3a–f** were synthesized (Table 2).

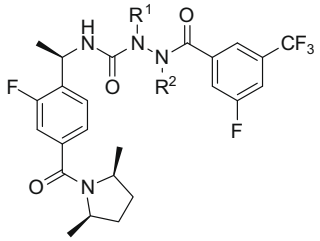
Table 1
B_1 receptor antagonist activity of benzamides **2a–g**



Compound	Amine	R^1	R^2	X	CAM IC_{50}[a] (nM)
2a	NHEt	H		CH	>10,000
2b	NEt_2	H		CH	3100
2c	N^iPr_2	H		CH	500
2d		H		CH	125
2e		H		CH	5.0
2f		F		CH	1.3
2g		F		N	28

[a] Numerical average of at least two experiments.

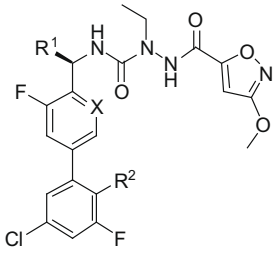
Table 2
B_1 receptor antagonist activity of semicarbazides **3a–f**

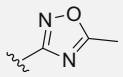
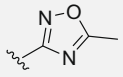
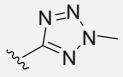
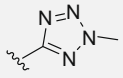
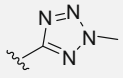


Compound	R^1	R^2	CAM IC_{50}[a] (nM)
3a	H	H	>1000
3b	Me	Me	>1000
3c	H	Et	>1000
3d	Et	H	1.0
3e	Me	H	4.4
3f	*i*-Pr	H	15

[a] Numerical average of at least two experiments.

Table 3
Antagonistic activity of semicarbazides **4a–e**



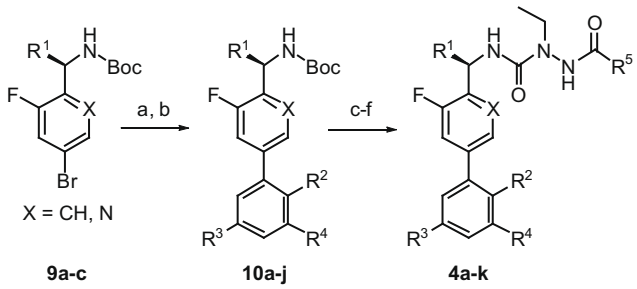
Compound	R^1	X	R^2	CAM IC_{50}, nM[a]	Efflux ratio[b]
4a	H	CH		0.43	2.0
4b	H	N		0.26	4.7
4c	H	CH		0.13	3.8
4d	H	N		0.20	7.2
4e	CH_3	N		0.14	4.6

[a] Numerical average of at least two experiments.
[b] Caco-2 directional transport ratio (B to A)/(A to B).

We found that the potency of both the N-unsubstituted semicarbazide **3a,** as well as the bis-*N*-methylated analogue **3b,** was less than IC_{50} = 1000 nM. Installation of an ethyl group at the R^2 position (**3c,** IC_{50} >1000 nM) did not lead to any measurable activity at the B_1 receptor. In contrast, a highly active compound was obtained (**3d**, IC_{50} = 1.0 nM) when R^1 only was an ethyl group. Further evaluation of the R^1 alkyl substituent demonstrated that methyl (**3e**, IC_{50} = 4.4 nM) and isopropyl (**3f**, IC_{50} = 15 nM) substitution led to lower activity compared to **3d,** suggesting the ethyl moiety represents an optimal small alkyl substituent for R^1 in this series. Unfortunately, this class of compounds (**3a–f**) proved to be compromised by low human microsomal stability, as for benzamides **2a–g**.

To improve the microsomal stability, the biaryl core was revisited and SAR of the corresponding semicarbazide derivatives was undertaken (Table 3). A 3-methoxyisoxazole-5-carbonyl group was incorporated adjacent to the semicarbazide, based on the observation that in similar biaryl cyclopropyl amino acid-based antagonists, this group was found to be optimal.[8] The general synthesis of the compounds is depicted in Scheme 2. Transformation of phenyl or pyridine bromides **9**[7,16] gave rise to the respective pinacol boron esters which were coupled with the selected aryl bromides[8,16,12] to furnish the biaryl compounds **10**. The final compounds **4a–k** were obtained by removal of the Boc group and subsequent HATU coupling with the corresponding carboxylic acids.

Compound **4a,** with an oxadiazole ring attached to the distal ring of the biaryl moiety, showed very good activity in the subnanomolar range (IC_{50} = 0.43 nM). A Caco-2 efflux ratio[17] of 2.0 indicates that the compound is likely to be a weak P-gp substrate. Activity, as well as solubility, could be increased by switching to the pyridine derivative **4b** (IC_{50} = 0.26 nM). However, in the pyridine series, Caco-2 efflux was higher as also shown for the tetrazoles **4c** and **4d**. This effect could be reduced to some extent by

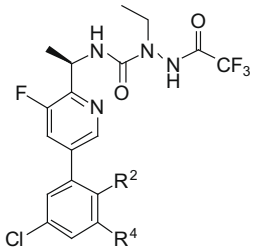


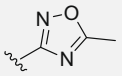
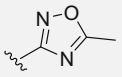
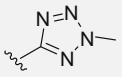
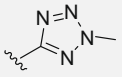
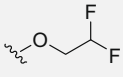
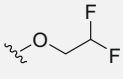
Scheme 2. Reagents and conditions: (a) bis(pinacolato) diborane, $Pd(dppf)Cl_2$, KOAc, DMF, 80 °C; (b) aryl bromide, $Pd(dppf)Cl_2$, K_2CO_3, DMF, 80 °C; (c) HCl/methanol; (d) $COCl_2$, *N*′-ethyl-hydrazinecarboxylic acid *tert*-butyl ester, DIPEA, THF; (e) HCl/Methanol; (f) R^5CO_2H, HATU, DIPEA, DMF.

the introduction of a methyl group into the benzyl position (**4e**, ratio 4.6 vs **4d**, ratio 7.2).

Further exploration of more than 70 different aliphatic and heterocyclic amide derivatives revealed that the trifluoroacetic acid (TFA) amide—present in **1**—served as the best functional group with respect to activity and Caco-2 efflux. In Table 4, representative examples of semicarbazides with the TFA amide are shown. In both classes with the oxadiazole and the tetrazole moiety, the compounds with a Cl/F-substitution pattern (**4g**, IC_{50} = 0.48 nM and **4i**, IC_{50} = 0.28 nM) were found to be more active than those with Cl/Cl-substitution (**4f**, IC_{50} = 1.3 nM and **4h**, IC_{50} = 0.64 nM). However, **4g** and **4i** still showed marginal Caco-2 efflux ratios of 2.4 and 3.9, respectively. Inspired by researchers from Merck,[8] we introduced a difluoroethyl ether into the phenyl ring of the semicarbazide derivatives. For the compounds with the Cl/F (**4k**,

Table 4
Antagonistic activity of semicarbazides **4f–k**



Compound	R^4	R^2	CAM IC_{50}[a] (nM)	Efflux ratio
4f	Cl		1.3	n.d.
4g	F		0.48	2.4
4h	Cl		0.64	n.d.
4i	F		0.28	3.9
4j	Cl		1.5	1.2
4k	F		1.8	1.1

[a] Numerical average of at least two experiments.

Table 5
Pharmacokinetic data for selected compounds (Wistar rat)

Compound	$t_{1/2}$[a] (min)	CL[a] (mL/min/kg)	F[b] (%)
4b	24	14	>45
4c	93	5	20
4j	135	7	>44
4k	121	2	50

[a] 1 mg/kg iv.
[b] 1 mg mg/kg po.

IC_{50} = 1.8 nM) and the Cl/Cl (**4j**, IC_{50} = 1.5 nM) pattern, the activity as well as the Caco-2 efflux values, were found to be excellent.

Pharmacokinetic data for selected compounds in male Wistar rats are shown in Table 5.[18] Compound **4b** revealed a satisfactory PK profile as it is modestly cleared after iv administration (CL = 14 mL/min/kg) and has an oral bioavailability of F >45%. Compound **4c** showed a better terminal plasma half life after iv administration ($t_{1/2}$ = 93 min) and lower clearance but oral bioavailability proved to be only 20%. The difluoro ethyl ether analogues **4j** and **4k** exhibited the best PK profiles in rat with low clearances (CL $\leqslant$ 7 mL/min/kg), long plasma half lifes ($t_{1/2}$ > 120 min) and good oral bioavailabilities ($F \geqslant$ 44%). There was no indication of any toxicity of semicarbazides in a cytotoxicity assay in human hepatocytes up to a concentration of 100 μM[19] and no mutagenic liability was determined in an Ames test.[20]

In summary, we describe the synthesis of two series of bradykinin B_1 receptor antagonists. The benzamide series showed high potency but suffered from low microsomal stabilities. This issue was resolved by the introduction of the semicarbazide functionality in combination with a biaryl scaffold. The resulting compounds (e.g., **4j–k**) expressed high B_1 receptor antagonistic activity, low Caco-2 efflux ratios, and excellent pharmacokinetic profiles.

Acknowledgements

We thank Pericles Calias and Brigitte Hoch for proofreading the manuscript and for their consent to handle any future correspondence. We also thank Jessica Bald, Anja Borrmann, Antje Burian, Anett Hauser, Birgit Hollmann, Bianka Knopp, Stephanie Köpke, Ricarda Lange, Nicole Liebelt, Corinna Mewes, Daniel Ohlendorf, Katy Pesta, Frank Polster, Dagmar Riexinger, Beatrice Rosskopp, Jana Rother, Edith Weigt, Rocco Weise, Daniela Wulf and Ariane Zwintscher for their skillful technical assistance.

References and notes

1. Moreau, M. E.; Garbacki, N.; Molinaro, G.; Brown, N. J.; Marceau, F.; Adam, A. *J. Pharmacol. Sci.* **2005**, 6.
2. Regoli, D.; Barabe, J. *Pharmacol. Rev.* **1980**, *32*, 1.
3. Marceau, F.; Hess, J. F.; Bachvarov, D. R. *Pharmacol. Rev.* **1998**, *50*, 357.
4. Calixto, J. B.; Medeiros, R.; Fernandes, E. S.; Ferreira, J.; Cabrini, D. A.; Campos, M. M. *Br. J. Pharmacol.* **2004**, *348*, 803.
5. Leeb-Lundberg, L. M. F.; Marceau, F.; Mueller-Esterl, W.; Pettibone, D. J.; Zuraw, B. L. *Pharmacol. Rev.* **2005**, *90*, 8234.
6. Kuduk, S. D.; Bock, M. G. *Curr. Top. Med. Chem.* **2008**, *8*, 1420.
7. Kuduk, S. D.; Di Marco, C. N.; Chang, R. K.; Wood, M. R.; Schirripa, K. M.; Kim, J. J.; Wai, J. C.; DiPardo, R. M.; Murphy, K. L.; Ransom, R. W.; Harrell, C. M.; Reiss, D. R.; Holahan, M. A.; Cook, J. L.; Hess, J. F.; Sain, N.; Urban, M. O.; Tang, C.; Prueksaritanont, T.; Pettibone, D. J.; Bock, M. G. *J. Med. Chem.* **2007**, *50*, 272.
8. Feng, D.-M.; DiPardo, R. M.; Wai, J. M.; Chang, R. K.; Di Marco, C. N.; Murphy, K. L.; Ransom, R. W.; Reiss, D. R.; Tang, C.; Prueksaritanont, T.; Pettibone, D. J.; Bock, M. G.; Kuduk, S. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 682.
9. Kuduk, S. D.; DiPardo, R. M.; Chang, R. K.; DiMarco, C. N.; Murphy, K. L.; Ransom, R. W.; Reiss, D. R.; Tang, C.; Prueksaritanont, T.; Pettibone, D. J.; Bock, M. G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3608.
10. Wood, M. R.; Schirripa, K. M.; Kim, J. J.; Kuduk, S. D.; Di Marco, C. N.; Chang, R. K.; Wai, J. C.; DiPardo, R. M.; Wang, B. L.; Cook, J. L.; Holahan, M. A.; Lemaire, W.; Mosser, S. D.; Bednar, R. A.; Wallace, A. A.; Mei, Q.; Yu, J.; Bohn, D. C.; Clayton, F. D.; Adarayan, E. A.; Sitko, G. R.; Leonard, Y. M.; Murphy, K. L.; Ransom, R. W.; Harrell, C. M.; Tang, C.; Prueksaritanont, T.; Pettibone, D. J.; Bock, M. G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 716.
11. Kuduk, S. D.; Chang, R. K.; Dipardo, R. M.; Di Marco, C. N.; Murphy, K. L.; Ransom, R. W.; Reiss, D. R.; Tang, C.; Prueksaritanont, T.; Pettibone, D. J.; Bock, M. G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5107.
12. Menzel, K.; Machrouhi, F.; Bodenstein, M.; Alorati, A.; Cowden, C.; Gibson, A. W.; Bishop, B.; Ikemoto, N.; Nelson, T. D.; Kress, M. H.; Frantz, D. E. *Org. Process Res. Dev.* **2009**, *13*, 519.
13. (a) Dyker, H.; Scherkenbeck, J.; Gondol, D.; Goehrt, A.; Harder, A. *J. Org. Chem.* **2001**, *66*, 3760; (b) Bailey, M. D.; Halmos, T.; Goudreau, N.; Lescop, E.; Llinas-Brunet, M. *J. Med. Chem.* **2004**, *47*, 3788; (c) Melendez, R. E.; Lubell, W. D. *J. Am. Chem. Soc.* **2004**, *126*, 6759.
14. Determined as described in: Kunz, W.; Gieschen, H. *Drug. Metab. Dispos.* **1998**, *26*, 1120. Microsomal preparations from different species were obtained from Tebu-bio, Offenbach, Germany.
15. Gibson, C.; Goodman, S. L.; Hahn, D.; Hölzemann, G.; Kessler, H. *J. Org. Chem.* **1999**, *64*, 7388.
16. Kuduk, S. D.; Wood, M. R. WO Patent 2006/132837, 2006; *Chem. Abstr.* **2006**, *143*, 133188.
17. Absorption systems express bidirectional permeability assay through Caco-2 cell monolayers: Artursson, P.; Palma, K.; Luthmanb, K. *Adv. Drug Delivery Rev.* **2001**, *46*, 27; Sambuy, Y.; De Angelis, I.; Ranaldi, G.; Scarino, M. L.; Stammati, A.; Zucco, F. *Cell Biol. Toxicol.* **2005**, *21*, 1.
18. The compounds were administered to male Wistar rats at 1 mg/kg iv (group 1) and 1 mg/kg. po (group 2). Aliquots of plasma samples were precipitated with methanol containing a structural analogue of the analyte as internal standard and analyzed by HPLC–MS/MS to determine time/concentration values. The pharmacokinetic parameters were calculated by using Winnonlin 5.1.
19. Cell viability assay with human plateable cryopreserved hepatocytes. Incubation was performed with different concentrations of the semicarbazide for 24 h at 37 °C with resazurin as substrate and detection of resorufin as reaction product by fluorimetry: Nociari, M. M.; Shalev, A.; Benias, P.; Russo, C. *J. Immunol. Meth.* **1998**, *213*, 157.
20. Pre-incubation test using *salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102. The assay was conducted with and without metabolic activation by S9. No toxic effects were noted at any concentration of the test item (up to 100 μM) in any of the tester strains. This means that no gene mutations by base pair changes or frameshifts in the genome of the tester strains were caused: Claxton, L. D.; Allen, J.; Auletta, A.; Mortelmans, K.; Nestmann, E.; Zeiger, E. *Mutat. Res.* **1987**, *189*, 83.