Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1584–1589

Solid phase synthesis and SAR of small molecule agonists for the GPR40 receptor

Stephen C. McKeown,[a,*] David F. Corbett,[a] Aaron S. Goetz,[b] Thomas R. Littleton,[b] Eric Bigham,[b] Celia P. Briscoe,[c,†] Andrew J. Peat,[c] Steve P Watson[a] and Deirdre M. B. Hickey[d]

[a]*Molecular Discovery Research, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*
[b]*Molecular Discovery Research, GlaxoSmithKline Research & Development, 5 Moore Drive, Research Triangle Park, NC 27709, USA*
[c]*Metabolic Centre of Excellence for Drug Discovery, GlaxoSmithKline Research & Development, 5 Moore Drive, Research Triangle Park, NC 27709, USA*
[d]*Infectious Diseases Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*

Received 26 October 2006; revised 21 December 2006; accepted 26 December 2006
Available online 4 January 2007

Abstract—The discovery, synthesis and structure–activity relationship (SAR) of novel carboxylic acid agonists for GPR40 are described. Aryl propionic acid **1**, identified from a high throughput screen, was selected for chemical exploration. Compound **2** was identified as our lead molecule through efficient solid phase combinatorial array chemistry and had an attractive in vitro and in vivo pharmacokinetic profile in rat. These ligands may prove useful in establishing a role for GPR40 in insulin regulation.

GPR40, GPR41 and GPR43 belong to a family of G-protein coupled receptors (GPCRs) that have been shown to be activated by free fatty acids.[1–6] GPR40 is activated by long chain fatty acids (e.g., linoleic and palmitic acids), whereas GPR41 and GPR43 are both activated by short chain fatty acids (e.g., acetic and propionic acid). GPR40 receptor mRNA has been reported to be expressed in human and rodent pancreatic islets[2,3,7] and it is well established that free fatty acids play a role in maintaining basal insulin secretion and to 'prime' the islet β-cells to respond to glucose following a prolonged fast.[8–12] A key role for GPR40 in pancreatic β-cells was suggested based on the siRNA downregulation of GPR40 expression in the mouse insulinoma cell line MIN6 which resulted in a decrease of the linoleic acid potentiation of insulin secretion.[3,13,14] Furthermore, our group has demonstrated the enhancement of insulin secretion in MIN6 cells by the selective GPR40 agonist (**2**) described in this paper.[15] Recent evidence also suggests that voltage-gated K^+ channel regulation of insulin release in rat pancreatic β-cells is mediated through the GPR40 receptor.[16,17] These data suggest that a small molecule GPR40 ligand could help regulate insulin secretion and as such present GPR40 as a potential target for Type II Diabetes. The discovery of selective tool molecules, both antagonists and agonists, will help us to further understand the role of this receptor.

We have recently reported the discovery of the aryl propionic acid **1** and the chemical optimisation of a subsequent analogue **2** as potent GPR40 agonists.[18] We describe here the solid phase combinatorial synthesis and initial SAR of the hit compound **1** that led to the discovery of compound **2**, Figure 1.

A high throughput screen of the GlaxoSmithKline compound collection yielded the aryl propionic acid **1** as a hit with a potency of pEC_{50} 6.3 in the GPR40 FLIPR assay.[15] Aryl propionic acid **1** produces a similar maximal response to linoleic acid ($pEC_{50} = 5.6$) indicating that it is a full agonist for the GPR40 receptor (compared to linoleic acid).

Keywords: GPR40 agonist; Diabetes; Solid phase chemistry.
* Corresponding author. Tel.: +44 1279 627558; fax: +44 1279 87 5414; e-mail: Steve.C.McKeown@gsk.com
† Present address: Diabetes Biology Department, Pfizer, La Jolla, San Diego, CA 92121, USA.

0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2006.12.084

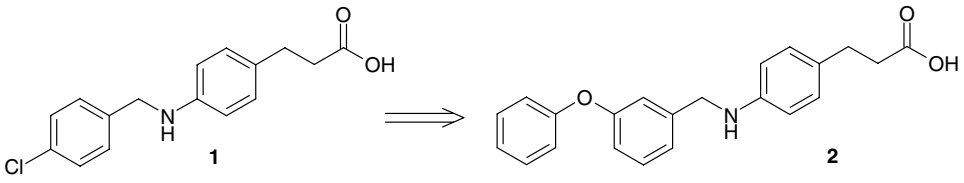
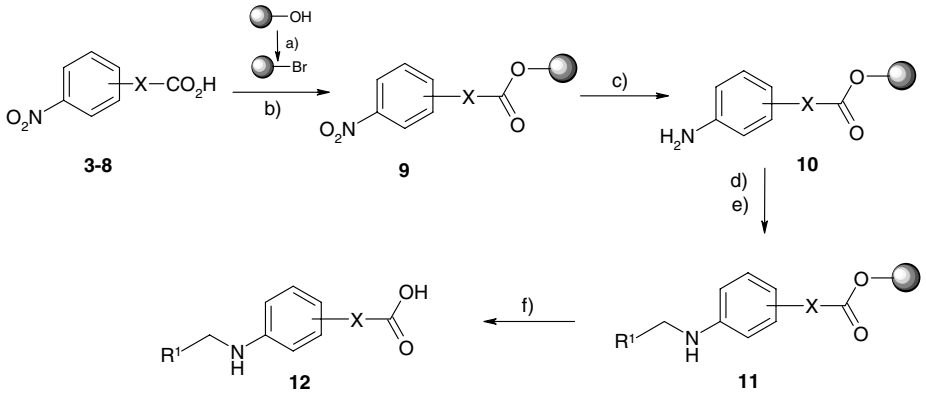


Figure 1. Optimisation of GPR40 agonist hit **1**.



Scheme 1. Reagents and conditions: (a) Ph_3PBr_2, CH_2Cl_2, 3 h, rt; (b) CsI, DIPEA, DMF, rt, 20 h, 70–88%; (c) $SnCl_2 \cdot 2H_2O$, NMP, 67 h, rt; (d) R^1CHO, 1% CH_3CO_2H, DMF, 2 h, rt; (e) $NaCNBH_3$, rt, 20 h and (f) TFA (33%), H_2O (2.5%), CH_2Cl_2, rt, 2 h, 20–75%.

Aryl propionic acid **1** exhibited good aqueous solubility (0.83 mg/mL), a low molecular weight (289 Da) and a clog *P* of 3.6. In addition, **1** was an attractive molecule for chemical optimisation since it can be assembled with relatively simple chemistry allowing for rapid exploration of the SAR. A chemical programme was therefore initiated with the aim of enhancing the potency and exploring the SAR of **1**. To achieve this, we chose to simultaneously vary two regions of the template exploiting the carboxylic acid as a solid phase attachment moiety to facilitate combinatorial array chemistry, Scheme 1 (Table 1).

The building blocks for the chemical array consisted of 6 nitro acids and 32 aromatic aldehydes (commercially available). These reagents were selected to explore length and regiochemistry of the carboxylic acid head group (Fig. 2) together with varying substituents in the lipophilic aromatic tail whilst producing compounds with a molecular weight below 400 and a clog *P* less than 7.

Table 1. Profile of HTS hit compound **1**

Parameter	Value
GPR40 pEC_{50}–FLIPR assay[15]	6.3 (100%)
GPR40 pEC_{50}–reporter assay[15]	6.0 (111%)
Solubility at pH 7.4 (mg/mL)	0.83
clog *P*	3.6
log *D* at pH 7.4	0.98
M_w	289

The nitro acids (**3**–**8**) were first coupled to the functionalised Wang-Br resin by using CsI and DIPEA (Scheme 1). Reduction of resin bound nitro product **9** was then effected with $SnCl_2$. The reductive alkylation of **10** was carried out by first forming the imine with the aldehyde, followed by the addition of sodium cyanoborohydride. These reactions were performed in a combinatorial manner using the IRORI directed sorting process.[19] The solid bound anilines (**10**) were placed in MicroKans™ (microreactors from IRORI) (0.04 mmol/reactor) with a radiofrequency tag, and then reacted with different aldehydes in parallel such that each aniline was reacted with every aldehyde in different microreactors. At each stage of the synthesis resin washings were carried out using a variety of solvents such as CH_2Cl_2, DMF, THF and H_2O. Final compound cleavage from the resin (**11**) was achieved using 33% TFA, 2.5% H_2O in CH_2Cl_2. Any N-Boc or *tert*-butyl protecting groups present in the

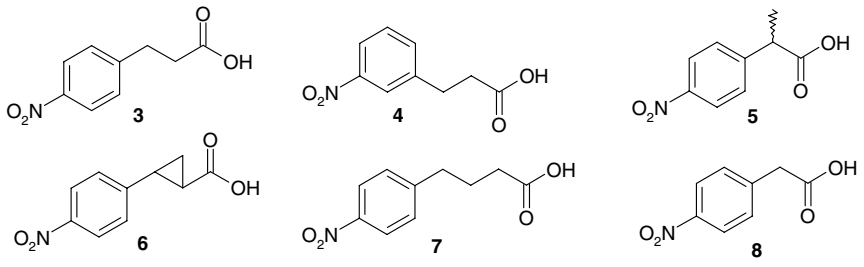


Figure 2. Nitro acid building blocks.

molecules (**11**) were also removed during the cleavage step. Those compounds that showed <85% purity by UV (HPLC) were purified by preparative HPLC, otherwise the cleaved dry products (**12**) were assayed directly.

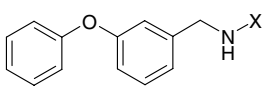
In total 167 compounds were submitted for GPR40 testing (not all data are shown). A significant increase in GPR40 potency was obtained (e.g., compound **2**) and clear SAR was defined (Table 2).

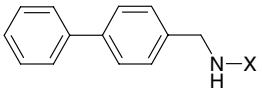
Table 2 shows the variation of the acid head group when combined with two of the preferred lipophilic tails. A 2-carbon spacer between the aryl and the carboxylic acid appeared to be important in maintaining GPR40 potency and efficacy with the original linear propionic acid (entries **2** and **19**) and cyclopropyl chains (entries **14** and **20**) being optimal. Homologation or shortening of the chain (entries **16**–**18** and **22**–**24**) led to a drop in potency. Interestingly, the 3-regioisomer **15** and **21** retained potency and appeared to possess higher intrinsic activities.

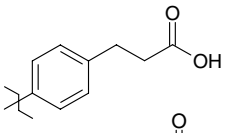
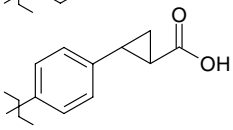
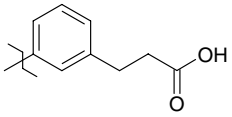
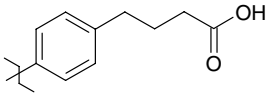
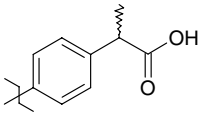
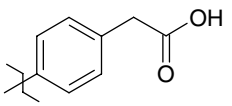
Table 3 shows the variation of R^1 when partnered with the optimal phenylpropionic acid head group. In general it can be seen that a lipophilic group at R^1 is preferred since any polar substituent results in the loss of potency. The favoured lipophilic groups are 3-phenoxy and 4-phenyl. These groups were consistently more potent than other lipophilic groups even when partnered with the alternative non-optimal acidic head groups.

Compound **2** was found to be inactive against family members GPR41 and GPR43, Table 4. This result is not surprising given that GPR40 is activated by long chain fatty acids such as lineolic acid, and GPR41 and GPR43 are activated by short chain fatty acids such as acetic and propionic acid. Furthermore, compound **2** was selective over a range of other fatty acid receptors, such as S1P1, HM74A, PPARα, δ and γ, and the prostaglandin receptors, EP_1, EP_3 and EP_4. The detailed pharmacological characterization and broader selectivity of compound **2** has previously been described by our group.[15]

Table 2. GPR40 SAR of the acid head groups

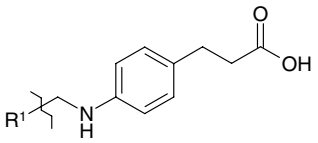




X	Compound	GPR40 pEC_{50} (% Max)[c]	Compound	GPR40 pEC_{50} (% Max)[c]
	**2**	7.1 ± 0.2 (69 ± 10)[b]	**19**	6.6 ± 0.2 (111 ± 6)[a]
	**14**	7.3 ± 0.1 (74 ± 14)[b]	**20**	7.4 ± 0.1 (80 ± 13)[b]
	**15**	6.4 ± 0.1 (148 ± 17)[a]	**21**	6.3 ± 0.1 (160 ± 7)[a]
	**16**	5.5 ± 0.1 (74 ± 24)[a]	**22**	5.8 ± 0.1 (62 ± 5)[a]
	**17**	4.78 ± 0.4 (34 ± 5)[a]	**23**	<4.5 (21 ± 1)[a]
	**18**	5.4 ± 0.1 (32 ± 5)[a]	**24**	4.91 ± 0.6 (69 ± 7)[a]

[a] pEC_{50} values are the average of two experiments.
[b] pEC_{50} values are the average of four experiments.
[c] Reporter gene assay expressing GPR40 in a CHO cell line.[2]

Table 3. GPR40 SAR of the lipophilic tails R^1



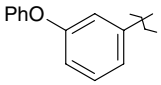
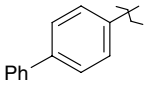
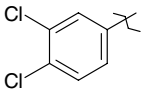
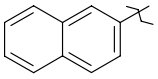
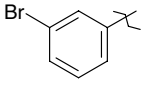
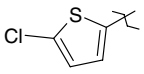
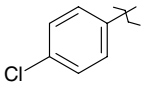
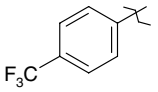
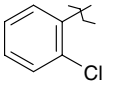
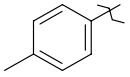
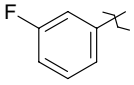
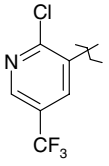
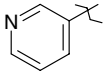
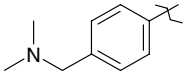
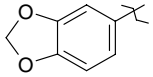
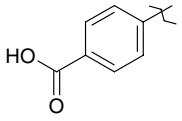
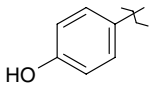
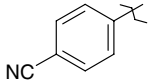
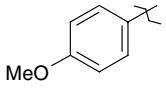
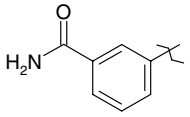
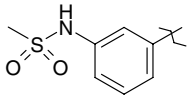
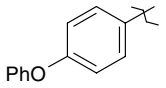
Compound	R^1	GPR40 pEC_{50} (% Max)[c]
2		7.1 ± 0.2 (69 ± 10)[b]
19		6.6 ± 0.2 (111 ± 6)[a]
25		6.6 ± 0.4 (117 ± 25)[b]
26		6.1 ± 0.1 (123 ± 21)[a]
27		6.1 ± 0.1 (120 ± 27)[a]
28		5.7 ± 0.03 (143 ± 34)[a]
1		6.0 ± 0.3 (111 ± 18)[b]
29		5.7 ± 0.1 (133 ± 5)[a]
30		5.4 ± 0.05 (122 ± 1)[a]
31		5.4 (152)[d]
32		5.3 ± 0.1 (120 ± 1)[a]
33		5.3 ± 0.2 (112 ± 0.4)[a]
34		5.1 ± 0.4 (144 ± 11)[b]
35		<4.5 (4 ± 1)[a]
36		<4.5 (12 ± 2)[a]

Table 3. (*continued*)

Compound	R^1	GPR40 pEC_{50} (% Max)[c]
37		<4.5 (141 ± 23)[b]
38		<4.5 (9 ± 3)[a]
39		<4.5 (37 ± 13)[a]
40		<4.5 (49 ± 2)[a]
41		5.9 ± 0.1 (159 ± 2)[a]
42		<4.5 (18 ± 2)[a]
43		<4.5 (4 ± 1)[a]
44		<4.5 (9 ± 2)[a]
45		6.71 (139)[d]

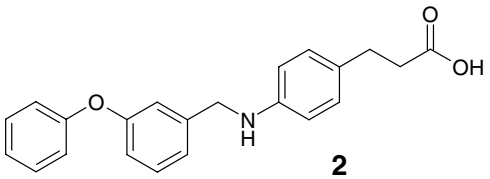
[a] pEC_{50} values are the average of two experiments.
[b] pEC_{50} values are the average of at least four experiments.
[c] Reporter gene assay expressing GPR40 in a CHO cell line.[2]
[d] Single experiment.

Table 4. Selectivity of compound **2**

Receptor	pEC_{50}	Receptor	fpK_i
GPR40	7.3	PPARα	<5.5
GPR41	<4.3	PPARδ	<5.5
GPR43	<4.3	PPARγ	<5.5
S1P1	<4.5	EP_1	<5.8
HM74A	<5.2	EP_3	6.4
		EP_4	<5.5

Table 5 shows the physicochemical properties and DMPK (in vitro and in vivo) data for compound **2**. Compound **2** had moderate aqueous solubility of 0.29 mg/mL (at pH 7.4), a molecular weight of 347 and a measured log*D* of 1.95 (at pH 7.4). The compound showed no inhibition of the CYP450 isoforms tested (1A2, 2C9, 2C19, 2D6 and 3A4 all >33 μM) and had acceptable microsomal stability with clearances of 2.3 (rat) and 0.74 (human) mL/min/g liver. As is common with lipophilic acids, compound **2**

Table 5. In vitro DMPK and Physicochemical profile of **2**



2

Parameter	Value	In vivo rat profile[b]	
		Parameter	Value
Solubility at pH 7.4 (mg/mL)	0.29	Cl (mL/min/kg)	24.9
CYP450[a], IC_{50} (μM)	1A2, 2C9, 2C19, 2D6, 3A4 all >33	V_{dss} (L/kg)	4.7
Microsomal clint (mL/min/g liver)	2.3 rat; 0.74 mouse	$t_{1/2}$ (po) (h)	5.9
Permeability (nm/sec)[c]	599	$t_{1/2}$ (iv) (h)	5.3
log *D* at pH 7.4	1.95	Bioavailability (%)	65
M_w	347		
Protein binding[d](%)	>99.6		

[a] In vitro CYP450 assay results using Gentest protocol.
[b] Average of three experiments. Doses = 3.9 mg/kg po; 1.0 mg/kg iv.
[c] Permeability measured using MDCK type I (low Pgp expressing) cell line.[20]
[d] Ultrafiltration assay using rat plasma.

showed a high level of protein binding (>99.6%) in the ultrafiltration assay using rat plasma. Compound **2** displayed good pharmacokinetics in rat with a good half-life (5.9 and 5.3 h after oral and iv administration, respectively), moderate clearance (24.9 mL/min/kg) and a bioavailability of 65%.

In summary, we have described the discovery of **1** through a high throughput screen and its optimisation using expedient solid phase chemical array technology to yield a potent lead molecule (**2**) for our GPR40 programme. Compound **2** showed greater than 500-fold selectivity for GPR40 over GPR41 and GPR43 and possessed a good in vitro and in vivo profile with excellent bioavailability. The chemical optimisation[18] and pharmacology[15] of **2** has previously been reported by our researchers. Further investigations of this series of molecules will be reported in due course.

Acknowledgments

We thank Mary Wells-Knecht, Zhiyang Zhao and Beth Beaudet for generating the DMPK data.

References and notes

1. Brown, A. J.; Jupe, S.; Briscoe, C. P. *DNA Cell. Biol.* **2005**, *24*, 54.
2. Briscoe, C. P.; Tadayyon, M.; Andrews, J. L.; Benson, W. G.; Chambers, J. K.; Eilert, M. M.; Ellis, C.; Elshourbagy, N. A.; Goetz, A. S.; Minnick, D. T.; Murdock, P. R.; Sauls, H. R. Jr.; Shabon, U.; Spinage, L. D.; Strum, J. C.; Szekeres, P. G.; Tan, K. B.; Way, J. M.; Ignar, D. M.; Wilson, S.; Muir, A. *J. Biol. Chem.* **2003**, *278*, 11303.
3. Itoh, Y.; Kawamata, Y.; Harada, M.; Kobayashi, M.; Fujii, R.; Fukusumi, S.; Ogi, K.; Hosoya, M.; Tanaka, Y.; Uejima, H.; Tanaka, H.; Maruyama, M.; Satoh, R.; Okubo, S.; Kizawa, H.; Komatsu, H.; Matsumura, F.; Noguchi, Y.; Shinohara, T.; Hinuma, S.; Fujisawa, Y.; Fujino, M. *Nature* **2003**, *422*, 173.
4. Kotarsky, K.; Nilsson, N. E.; Flodgren, E.; Owman, C.; Olde, B. *Biochem. Biophys. Res. Commun.* **2003**, *301*, 406.
5. Le Poul, E.; Loison, C.; Struyf, S.; Springael, J. Y.; Lannoy, V.; Decobecq, M. E.; Brezillon, S.; Dupriez, V.; Vassart, G.; Van Damme, J.; Parmentier, M.; Detheux, M. *J. Biol. Chem.* **2003**, *278*, 25481.
6. Nilsson, N. E.; Kotarsy, K.; Owman, C.; Olde, B. *Biochem. Biophys. Res. Commun.* **2003**, *303*, 1047.
7. Tomita, T.; Masuzaki, H.; Iwakura, H.; Fujikura, J.; Noguchi, M.; Tanaka, T.; Ebihara, K.; Kawamura, J.; Komoto, I.; Kawaguchi, Y.; Fujimoto, K.; Doi, R.; Shimada, Y.; Hosoda, K.; Imamura, M.; Nakao, K. *Diabetologia* **2006**, *49*, 962.
8. Stein, D. T.; Stevenson, B. E.; Chester, M. W.; Basit, M.; Daniels, M. B.; Turley, S. D.; Mcgarry, J. D. *J. Clin. Invest.* **1997**, *100*, 398.
9. Dobbins, R. L.; Chester, M. W.; Daniels, M. B.; Mcgarry, J. D.; Stein, D. T. *Diabetes* **1998**, *47*, 1613.
10. Dobbins, R. L.; Chester, M. W.; Stevenson, B. E.; Daniels, M. B.; Stein, D. T.; Mcgarry, J. D. *J. Clin. Invest.* **1998**, *101*, 2370.
11. Gravena, C.; Mathias, P. C.; Ashcroft, S. J. *J. Endocrinol.* **2002**, *173*, 73.
12. Steneberg, P.; Rubins, N.; Bartoov-Shifman, R.; Walker, M. D.; Edlund, H. *Cell Metab.* **2005**, *1*, 245.
13. Shapiro, H.; Shachar, S.; Sekler, I.; Herschfinkel, M.; Walker, M. D. *Biochem. Biophys. Res. Commun.* **2005**, *335*, 97.
14. Miyazaki, J.; Araki, K.; Yamato, E.; Ikegami, H.; Asano, T.; Shibasaki, Y.; Oka, Y.; Yamamura, K. *Endocrinology* **1990**, *127*, 126.
15. Briscoe, C. P.; Peat, A. J.; McKeown, S. C.; Corbett, D. F.; Goetz, A. S.; Littleton, T. R.; McCoy, D. C.; Kenakin, T. P.; Andrews, J. L.; Ammala, C.; Fornwald, J. A.; Ignar, D. M.; Jenkinson, S. *Br. J. Pharmacol.* **2006**, *148*, 619.
16. Gromada, J. *Endocrinology* **2006**, *147*, 672, and references therein.
17. Feng, D. D.; Luo, Z.; Roh, S.; Hernandez, M.; Tawadros, N.; Keating, D. J.; Chen, C. *Endocrinology* **2006**, *147*, 674.
18. Garrido, D. M.; Corbett, D. F.; Dwornik, K. A.; Goetz, A. S.; Littleton, T. R.; McKeown, S. C.; Mills, W. Y.;

Smalley, T. L.; Briscoe, C. P.; Peat, A. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1840.

19. (a) Xiao, X.; Li, R.; Zhuang, H.; Ewing, B.; Karunaratne, K.; Lillig, J.; Brown, R.; Nicolaou, K. C. *Biotechnol. Bioeng.* **2000**, *71*, 44; (b) For more information about this technology, see: www.irori.com.
20. Polli, J. W.; Humphreys, J. E.; Wring, S. A.; Burnette, T. C.; Read, K. D.; Hersey, A.; Butina, D.; Bertolotti, L.; Pugnaghi, F.; Serabjit-Singh, C. J. In *Progress in Reduction, Refinement, Replacement of Animal Experimentation*; Balls, M., van Zeller, A. M., Halder, M., Eds.; Elsevier Science: New York, 2000; p 271.