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Discovery of new PPARγ agonists based on arylopeptoids

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ABSTRACT

In this study we present the design, synthesis and biological evaluation of a small, first-generation library of small molecule aromatic amides based on the arylopeptoid skeleton. The compounds were efficiently synthesized using a highly convenient submonomer solid-phase methodology which potentially allows for access to great product diversity. The synthesized compounds were tested for their ability to activate peroxisome proliferator-activated receptors (PPARs) and they all acted as PPARγ agonists in the μM range spanning from 2.5- to 14.7-fold activation of the receptor. This is the first discovery of bioactive molecules based on the arylopeptoid architecture.

Metabolic disorders such as obesity, type 2 diabetes mellitus, insulin resistance and atherosclerosis are escalating globally at an epidemic rate. Although these so-called 'lifestyle diseases' are related to the balance between energy expenditure and energy intake, the underlying biochemistry and pharmacology is exceptionally complex and include the interaction and orchestration of numerous receptors such as the peroxisome proliferator-activated receptors (PPARs).[1] PPARs are ligand-activated transcription factors belonging to the nuclear receptor superfamily. PPARs are known to play an important role in the general transcriptional control of numerous cellular processes, including lipid metabolism, glucose homeostasis, cell cycle progression, cell differentiation, inflammation and extracellular matrix remodeling. Three subtypes of PPAR have been identified: PPARα, PPARβ/PPARδ and PPARγ, with PPARγ being the most extensively investigated isoform as well as the most highly expressed PPAR subtype in adipocytes and macrophages.[2] A number of small-molecule PPARγ agonists have been identified, comprising the antidiabetic thiazolidinediones (TZDs) rosiglitazone,[3] troglitazone,[4] farglitazar,[5] and non-TZD L-796449[6] (Fig. 1). Extensive SAR studies on PPARγ ligands can be summarized in a simplified pharmacophore model where chemical determinants such as an acidic head group, an aromatic linker and a hydrophobic (hetero)aromatic tail are key elements.[7] The acidic head group can form polar interactions with one subpocket of the PPARγ ligand binding site while the aromatic tail can bind to the second and highly hydrophobic subpocket.

Intriguingly, this pharmacophore model can be accommodated by the structure of N-substituted aminomethyl benzamides, or arylopeptoids, which is a novel class of aromatic oligoamides.[8] We have recently developed highly efficient solution-phase and solid-phase methodologies for 'submonomer' synthesis of arylopeptoids in which the arylopeptoid residues are created directly on the growing chain in an iterative manner by acylation-substitution cycles (Fig. 2). Only inexpensive and easily accessible acylation reagents derived from bromo- or chloromethylbenzoic acids are needed in the acylation steps and in principle, any imaginable primary amine may be used to install the side chain in the ensuing substitution steps. This methodology therefore gives facile access to products of highly diverse nature.

Only few applications of N-substituted aromatic oligoamides have been demonstrated to date: an example is the formation of crescent and helical structures in N-substituted benzanilides,[9] and the use of these as α-helix mimetics for inhibition of protein–protein p53–hMDM2 interactions.[10] Herein, we describe the discovery of bioactive compounds based on N-substituted aminomethyl benzamide backbones.

Inspired by the combination of backbone similarities with PPARγ agonists and our convenient synthetic methods we designed a first generation library of potential PPARγ agonists **2a–i** and **3a–i** consisting of an arylopeptoid skeleton (Scheme 2). Since a number of the existing PPARγ agonists contain a phenylpropionic acid moiety we decided to incorporate a *para*-substituted

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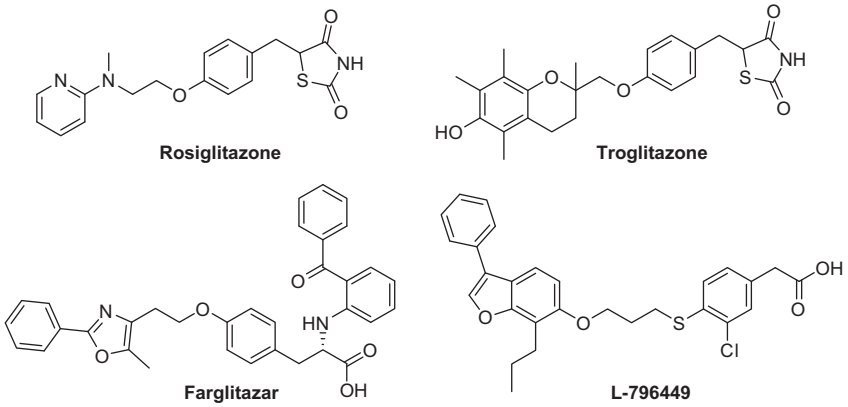


Figure 1. Selection of PPARγ agonists.

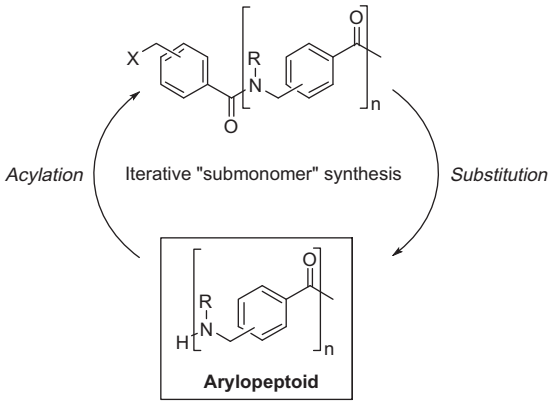
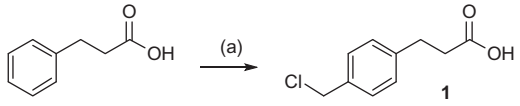


Figure 2. Arylopeptoids: N-substituted aminomethyl benzamides.



Scheme 1. Reagents and conditions: synthesis of **1**. Key: (a) formaldehyde (37% aq), HCl (g), $ZnCl_2$, 80 °C, 12 h.

phenylpropionic acid moiety at the C-terminus rather than a benzoic acid moiety. At the second residue the compounds carry either a *para*- or a *meta*-arylopeptoid residue in order to investigate the possible effects of substitution pattern on the agonist activities. Furthermore, three types of substituents were installed at the backbone nitrogens: a simple proton (obtained by acidic removal of 2,4-dimethoxybenzyl side chains), an ethyl or an isopropyl side chain. In our previous studies we have established that the amide group of the arylopeptoid backbone may exist as a *cis*/*trans* mixture which may be controlled by proper choice of side chains.[8] Thus, an increasing content of *cis* amide is obtained for simple alkyl side chains of increasing bulk and therefore, there will be a higher content of *cis* amide when installing isopropyl side chains than ethyl side chains which we speculated may effect the agonist efficiency.

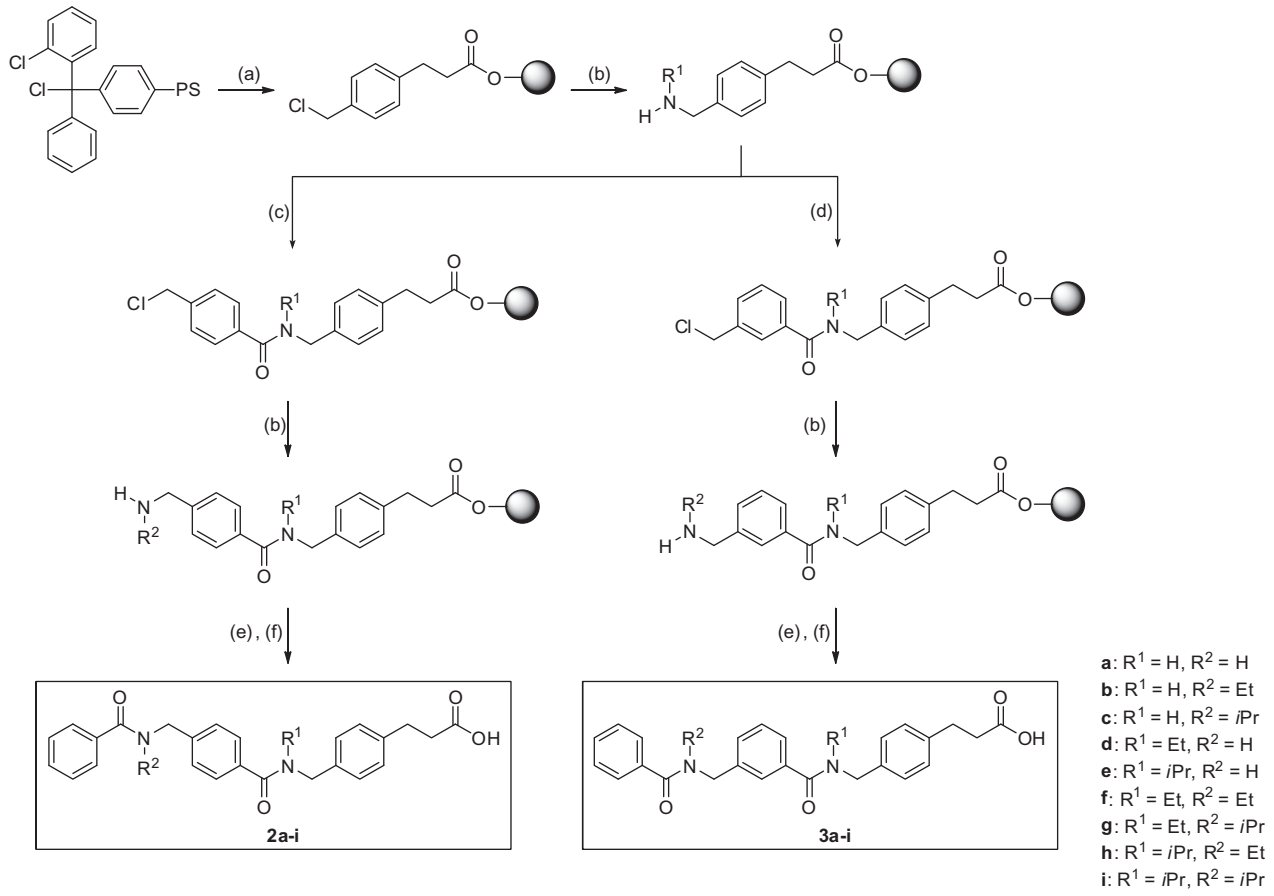
Prior to solid-phase synthesis of the designed library we thus only needed to synthesize the altered starting material 2-[4-(chloromethyl)phenyl]propanoic acid **1** which was obtained in 42% yield in one step by chloromethylation of 3-phenylpropanoic acid as described in the literature (Scheme 1).[11]

The designed library was then synthesized by direct adaptation of our previously described solid-phase methodology for submonomer synthesis of arylopeptoids based on the use of COMU (*O*-benzotriazolyl-*N*,*N*-tetramethyluronium hexafluorophosphate) in the substitution steps (Scheme 2).[8b] Thus, phenylpropionic acid **1** was first reacted with a 2-chlorotrityl chloride polystyrene resin in CH_2Cl_2 in the presence of DIPEA. The first side chain was then installed by reaction with the required primary amine (ethyl-, isopropyl- or 2,4-dimethoxybenzylamine) in DMSO at 50 °C. COMU-mediated acylation of the resulting secondary amine with either 3- or 4-chloromethylbenzoic acid followed by an additional substitution reaction with one of the three selected primary amines, and then capping with benzoylchloride completed the synthesis. Products containing 2,4-dimethoxybenzyl side chains (**2a**–**e** and **3a**–**e**) were cleaved off with TFA–H_2O (95:5) which resulted in concomitant removal of the 2,4-dimethoxybenzyl side chains thereby liberating the secondary amides while the remaining products (**2f**–**i** and **3f**–**i**) were cleaved off using HFIP–CH_2Cl_2 (1:4) as previously described. The desired products **2a**–**i** and **3a**–**i** were obtained in >97% HPLC purity in 33–42% yield (18–24 mg product) after preparative HPLC purification.

The synthesized compounds were analyzed for their ability to activate PPARγ. A mouse embryo fibroblast cell line was transiently transfected with a Gal4 responsive luciferase reporter and a plasmid encoding the fusion between the Gal4 DNA binding domain and the human PPARγ ligand binding domain. The system is very sensitive for identification of PPARγ agonists and activation of the luciferase reporter is indicative of ligand dependent stimulation of PPARγ. Rosiglitazone was used as a positive control for PPARγ activation. The PPARγ activating properties of the compounds were compared to both the DMSO vehicle and the rosiglitazone positive control and are depicted in Table 1 as fold activation over vehicle and relative activation compared to rosiglitazone. Screening was performed at 10 and 100 μM concentration. The synthesized compounds did not display cytotoxicity and were all PPARγ agonists in the μM range spanning from 2.5- to 14.7-fold activation of the receptor. However, no clear trend between structure and activity could be deduced.

An increased activity in the transactivation assay is only indicative of agonist activity. In order to measure binding to the receptor more directly we furthermore analyzed the ability of selected compounds to displace a known ligand as determined by an in vitro competitive binding assay using time-resolved fluorescence resonance energy transfer. A terbium labeled anti-GST antibody was used to label purified GST-tagged human PPARγ ligand binding domain. Energy transfer from terbium to the tracer, a fluorescent pan PPAR agonist, enabled read-out of each test compound's ability to displace the tracer. As seen from Table 2 the three tested compounds (**3a**, **3e** and **3f**) were all able to compete for binding to the PPARγ-LBD though with relatively low affinity.

PPARγ is a rather promiscuous receptor and the relatively large ligand binding pocket allow for association with a variety of



Scheme 2. Reagents and conditions: solid-phase submonomer synthesis of **2a–i** and **3a–i**. Key: (a) **1** (1.2 equiv, 0.14 M), DIPEA (6.0 equiv), CH_2Cl_2, rt, 1 h; (b) *R*-NH_2, (20 equiv, 2.0 M), DMSO, 50 °C, 1 h; (c) 4-($ClCH_2$)ArCOOH (3.0 equiv, 1.0 M), COMU (3.5 equiv), DIPEA (7.0 equiv), rt, 20 min; (d) 3-($ClCH_2$)ArCOOH (3.0 equiv, 1.0 M), COMU (3.5 equiv), DIPEA (7.0 equiv), rt, 20 min; (e) BzCl (4.0 equiv, 1.0 M), DIPEA (8.0 equiv), CH_2Cl_2, rt, 15 min; (f) Compounds **2a–e** and **3a–e**: TFA/water 95:5, rt, 1 h; **2f–i** and **3f–i**: HFIP–CH_2Cl_2 1:4, rt, 30 min.

Table 1
PPARγ activation of all synthesized compounds[a]

Compd	Fold above vehicle		Relative to rosiglitazone (%)	
	Avg.	Std.	Avg.	Std.
2a	2.5	0.6	5.4	1.4
3a	5.6	0.9	14.1	2.2
2b	6.0	1.5	13.1	3.2
3b	9.3	2.1	23.5	5.3
2c	5.7	1.4	12.5	3.0
3c	7.3	1.3	18.4	3.3
2d	6.2	1.6	11.8	3.0
3d	8.1	1.1	15.1	2.2
2e	12.6	1.3	21.2	2.2
3e	13.4	3.1	27.2	6.4
2f	7.3	0.5	13.8	0.9
3f	11.2	2.8	20.9	5.3
2g	3.6	1.5	6.9	2.9
3g	6.2	0.6	11.6	1.2
2h	6.9	0.9	11.6	1.4
3h	5.8	1.5	11.8	3.1
2i	14.7	2.5	24.7	4.2
3i	5.3	1.2	10.7	2.4

[a] Compounds were screened at 100 μM concentration.

compounds. Thus, it is not unusual for PPARγ to 'share' ligands with the family members PPARα and PPARβ/PPARδ or members of the family of obligate heterodimerization partners retinoid X receptors (RXRs). To evaluate the selected compounds' receptor selectivity they were tested in transactivation assays essentially identical to the PPARγ transactivation assay but with substitution of the PPARγ-LBD with PPARα-, PPARβ-/PPARδ- or RXRα-LBD. Among the selected compounds only **3a** specifically activated PPARγ, whereas **3e** and **3f** also activated RXRα and PPARα, respectively (Table 2).

Table 2
Evaluation of binding affinity of selected compounds

Compd	Ligand displacement (IC_{50}, μM)	PPARα activation	PPARδ activation	RXRα activation
3a	62.7	–	–	–
3e	38.7	–	–	++
3f	56.2	+	–	–

Signatures: –, no effect (less than twofold activation); +, activation (more than twofold, less than fivefold); ++, activation (more than fivefold, less than 10-fold).

It has been shown that thiazolidinediones such as rosiglitazone not only act as PPARγ agonists but also as free fatty acid receptor 1 (FFA1) agonists.[12] FFA1 responds to free fatty acids and increases glucose stimulated insulin secretion in pancreatic β-cells, and has recently also appeared as an interesting target for treatment of type 2 diabetes.[13] This prompted us to screen our ensemble of compounds towards FFA1 but no agonist or antagonist activity was observed at a 10 μM level (data not shown). These results indicate that this arylopeptoid-based compound series are selective towards PPAR with no cross-activation of FFA1.

In conclusion, we have used our previously developed solid-phase methodology for facile synthesis of a small, first-generation

library of small molecule aromatic amides based on an arylopeptoid backbone. The synthesized compounds did not display cytotoxicity and were all PPARγ agonists in the μM range spanning from 2.5- to 14.7-fold activation of the receptor. However, no clear trend between structure and activity could be deduced. Evaluation of three selected compounds' (**3a**, **3e** and **3f**) binding affinity showed that they were all able to compete for binding to the PPARγ-LBD although with moderate affinity. With respect to receptor selectivity, only **3a** exclusively activated PPARγ, whereas **3e** and **3f** also activated RXRα and PPARα, respectively. As this is the first discovery of bioactive arylopeptoids, further diversification of the compounds holds promise that more active and selective arylopeptoids can be developed.

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Supplementary data

Supplementary data (PPAR assays, FFA1 assays, synthetic procedures, and characterization data for all new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.05.034.

References and notes

1. (a) Haslam, D. W.; James, W. P. T. *Lancet* **2005**, *366*, 1197; (b) Wang, Y. C.; McPherson, K.; Marsh, T.; Gortmaker, S. L.; Brown, M. *Lancet* **2011**, *378*, 815; (c) Zimmet, P.; Alberti, K. G. M. M.; Shaw, J. *Nature* **2001**, *414*, 782.
2. The University of Sydney. Flavonoid PPAR Agonists WO 2,009,026,657; 2009.
3. Beecham Group p.l.c. Brentford, England, U.S. 5002953A; 1989.
4. Fujiwara, T.; Yoshioka, S.; Yoshioka, T.; Ushiyama, I.; Horikoshi, H. *Diabetes* **1988**, *37*, 1549.
5. Henke, B. R.; Blanchard, S. G.; Brackeen, M. F.; Brown, K. K.; Cobb, J. E.; Collins, J. L., ; Harrington, W. W., Jr.; Hashim, M. A.; Hull-Ryde, E. A.; Kaldor, I.; Kliewer, S. A.; Lake, D. H.; Leesnitzer, L. M.; Lehmann, J. M.; Lenhard, J. M.; Orband-Miller, L. A.; Miller, J. F.; Mook, R. A., Jr.; Noble, S. A.; Oliver, W., Jr.; Parks, D. J.; Plunket, K. D.; Szewczyk, J. R.; Willson, T. M. *J. Med. Chem.* **1998**, *41*, 5020.
6. Berger, J.; Leibowitz, M. D.; Doebber, T. W.; Elbrecht, A.; Zhang, B.; Zhou, G.; Biswas, C.; Cullinan, C. A.; Hayes, N. S.; Li, Y.; Tanen, M.; Ventre, J.; Wu, M. S.; Bergeri, G. D.; Mosleyi, R.; Marquisi, R.; Santinii, C.; Sahooi, S. P.; Tolmani, R. L.; Smith, R. G.; Moller, D. E. *J. Biol. Chem.* **1999**, *274*, 6718.
7. Lamers, C.; Schubert-Zsilavecz, M.; Merk, D. *Expert Opin. Ther. Pat.* **2012**, *22*, 803.
8. (a) Hjelmgaard, T.; Faure, S.; De Santis, E.; Staerk, D.; Alexander, B. D.; Edwards, A. A.; Taillefumier, C.; Nielsen, J. *Tetrahedron* **2012**, *68*, 4444; (b) Hjelmgaard, T.; Faure, S.; Staerk, D.; Taillefumier, C.; Nielsen, J. *Org. Biomol. Chem.* **2011**, *9*, 6832; (c) Hjelmgaard, T.; Faure, S.; Staerk, D.; Taillefumier, C.; Nielsen, J. *Eur. J. Org. Chem.* **2011**, 4121.
9. Tanatani, A.; Yokoyama, A.; Azumaya, I.; Takakura, Y.; Mitsui, C.; Shiro, M.; Uchiyama, M.; Muranaka, A.; Kobayashi, N.; Yokozawa, T. *J. Am. Chem. Soc.* **2005**, *127*, 8553.
10. Campbell, F.; Plante, J. P.; Edwards, T. A.; Warriner, S. L.; Wilson, A. J. *Org. Biomol. Chem.* **2010**, *8*, 2344.
11. Bogdanov, M. N. *J. Gen. Chem. USSR* **1958**, *28*, 1670.
12. (a) Kotarsky, K.; Nilsson, N. E.; Flodgren, E.; Owman, C.; Olde, B. *Biochem. Biophys. Res. Commun.* **2003**, *301*, 406; (b) Smith, N. J.; Stoddart, L. A.; Devine, N. M.; Jenkins, L.; Milligan, G. *J. Biol. Chem.* **2009**, *284*, 17527; (c) Nunez, E. A. *Prostaglandins Leukot. Essent. Fatty Acids* **1997**, *57*, 107.
13. Stoddart, L. A.; Smith, N. J.; Milligan, G. *Pharmacol. Rev.* **2008**, *60*, 405.