Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3187–3190

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Substituted Uracil Derivatives as Potent Inhibitors of Poly(ADP-ribose)polymerase-1 (PARP-1)

Henning Steinhagen,[a,*] Michael Gerisch,[a] Joachim Mittendorf,[a] Karl-Heinz Schlemmer[b] and Barbara Albrecht[c]

[a]*Institute of Medicinal Chemistry, Pharma Research Centre, Bayer AG, D-42096 Wuppertal, FRG*
[b]*Institute of Pharmacokinetics, Pharma Research Centre, Bayer AG, D-42096 Wuppertal, FRG*
[c]*Institute of Cardiovascular Research, Pharma Research Centre, Bayer AG, D-42096 Wuppertal, FRG*

Received 22 April 2002; revised 2 July 2002; accepted 19 July 2002

Abstract—A new class of PARP-1 inhibitors, namely substituted fused uracil derivatives were synthesised. Starting from a derivative with an $IC_{50} = 2\,\mu M$ the chemical optimisation program led to compounds with more than a 100-fold increase in potency ($IC_{50} < 20\,nM$). Additionally, physicochemical and pharmacokinetic properties were evaluated. It could be shown that compounds bearing a piperazine or phenyl substituted βAla-Gly side chain exhibited the best overall profile.

Introduction

The nuclear enzyme poly-(ADP-ribose) polymerase-1 (PARP-1), also known as poly-(ADP-ribose) synthetase (PARS), is involved in a variety of physiological processes related to DNA repair.[1] PARP-1 is activated by DNA strand breaks and uses nicotinamide dinucleotide (NAD) as a substrate for poly-ADP ribosylation of several proteins. The activation of PARP-1 plays an important role in *N*-methyl-D-aspartate (NMDA) and NO induced neurotoxicity,[2] cerebral ischemia[3] and ischemia/reperfusion injury.[4] PARP-1 is further involved in a variety of transcriptional mechanisms regulating inflammation and apoptosis.[1c] Thus, PARP-1 became an attractive target for possible clinical use and consequently, a variety of PARP-1 inhibitors were discovered. Many of the known inhibitors fall in the class of benzamides **I** (Fig. 1), which are structural analogues of NAD and are thought to compete with NAD at the catalytic domain.[5] Compounds **I** were shown to be active in the low micromolar to nanomolar range.[6] In general, cyclic derivatives of benzamides **I**, including dihydroisoquinolin-1-ones,[7] quinazolin-4-ones,[8] benzimidazoles,[9] phenanthridin-6-ones,[10] and 1,8-naphthylimides[11] showed an increased potency in PARP-1 inhibition with respect to benzamides. In this communication we wish to report the optimisation of a novel class of PARP-1 inhibitors, namely fused uracils **II** (Fig. 1).

This novel class of PARP-1 inhibitors could be identified by similarity searching in our corporate compound collection. One of the identified compounds was the trifluoromethyl substituted derivative **1** that showed an IC_{50} value of 2 μM. Based upon this promising finding a lead optimisation program was started with the goal to improve potency, physicochemical and pharmacokinetic (PK) properties.

Chemistry

In order to systematically optimise these properties an approach for the synthesis of the fused uracils **II** was developed that allowed a broad variation of both the *N*-side chain R^2 and also of the condensed (hetero) alkyl moiety. The key reaction in assembling the heterocyclic core was the cyclisation of an imine with chlorocarbonyl isocyanate[12] as exemplified in Scheme 1. Typically, the imine can be generated in situ by reacting the appropriate ketone with a primary amine followed by direct conversion in toluene at reflux to the desired uracils. Aqueous workup and purification by chromatography led to pure products. Based on this method nearly 900

*Corresponding author. Tel.: +49-202-36-5171; fax: +49-202-36-4624; e-mail: henning.steinhagen@aventis.com

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00602-9

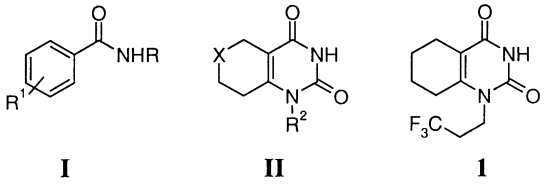
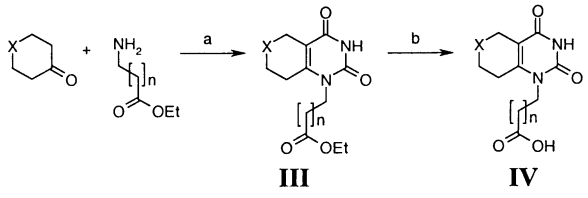


Figure 1. Substituted benzamides (**I**) and new uracil based (**II**, **1**) PARP-1 inhibitors.

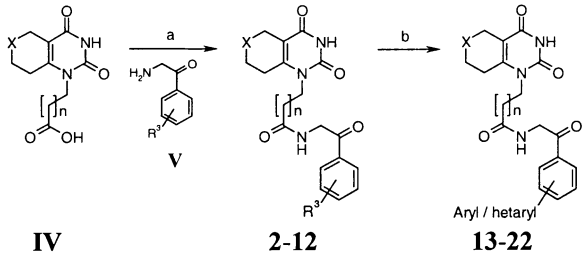
compounds were synthesised and screened for inhibition on PARP-1. The most interesting and potent derivatives of this series are discussed in this communication.

For most derivatives an amino alkanoate was used as the amine and the carboxylic acids **IV** could be obtained after saponification of **III** (Scheme 1). Subsequently, the acids **IV** were coupled with the primary amines **V** or **VI** to the corresponding amides **2**–**12** and **23**–**34** using standard conditions (Schemes 2 and 3). The amines **V** that were used for coupling can be prepared from the corresponding commercially available bromo acetophenones via amination according to published methods.[13] The piperazine substituted amines **VI** can be prepared by a simple two step sequence (acylation and amination) as exemplified in Scheme 3.

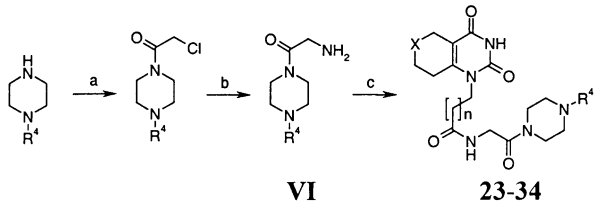
Additionally to the products that were directly accessible via amide formation, the aryl bromides **7**–**9** could be further derivatised to the biarylic compounds **13**–**22** using Pd catalysed Stille or Suzuki type cross couplings (Scheme 2). Compounds bearing spacer variations (Table 3) were prepared via similar methods.[14] Compound **44** was prepared using 1,8-nonadien-5-one and converted to **45** using by RCM reaction[15] with Grubbs catalyst (Scheme 4).



Scheme 1. (a) Campher sulfonic acid, toluene, reflux, 2 h, then Cl(CO)NCO, toluene, reflux, 1 h; (b) LiOH, ethanol/water, reflux, 1 h.



Scheme 2. (a) EDC, HOBt, DMAP, 4-methylmorpholine, DMF, rt, 18 h; (b) for **7**–**9** (R^3 = Br): aryl/hetaryl-$SnBu_3$, 5 mol% [$PdCl_2(PPh_3)_2$], DMF, 120 °C, 18 h, or aryl/hetaryl-$B(OH)_2$, 2 M Na_2CO_3, 5 mol% [$Pd(PPh_3)_4$], dioxane, 80 °C, 12 h.

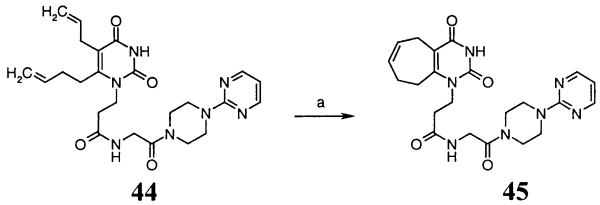


Scheme 3. (a) Chloroacetyl chloride, triethylamine, methylene chloride, 0 °C, 30 min; (b) hexamethylene tetramine, chloroform, rt, 18 h then ethanol, HCl, 50 °C, 3 h; (c) **IV**, EDC, HOBt, DMAP, 4-methylmorpholine, DMF, rt, 18 h.

Biological Results and Discussion

All compounds were fully characterised by ^{1}H NMR, MS and HPLC and have been tested as inhibitors for human recombinant PARP-1. Solubility was determined in phosphate buffered saline (1% DMSO) at pH 6.5 (24 h equilibration). IC_{50} and EC_{50} values were determined in dose–response inhibition[16] and cell protection assays.[17] The structure–activity relationships (SARs) of the novel PARP-1 inhibitors were systematically explored by varying both side chain R^2 and the (hetero) cyclohexene moiety. In the course of the studies it could be found that compounds bearing a ring system connected via a β-alanine-glycine (βAla-Gly) spacer gave an optimum in potency (Tables 1–3). The attached ring systems leading to most potent compounds were either substituted phenyls or piperazines. In addition, it was shown that a 3,6-dihydro-2-thiopyrane subunit (X = S) resulted in a three to tenfold increase in potency compared to a cyclohexenyl moiety (X = C).

In a first optimisation round the phenyl ring of **2** was widely varied (Table 1). Compared to **2** (IC_{50} = 450 nM) all of the given substituents led to an improvement in potency. The SAR regarding R^3 is somewhat flat with some EC_{50} values being higher than expected (e.g., **9**, **14**). This might be explained by a selective transport mechanism of the compounds for membrane passage. Regarding the overall profile (IC_{50}, EC_{50} and solubility) the biarylic compounds **19** and **20** proved to be an optimum. Since solubility was a crucial parameter upon optimisation (iv application anticipated) **20** (sol. = 305 mg/l) was considered for PK-studies. In order to further improve solubility the phenyl ring was replaced by the inherently better soluble piperazine ring (Table 2). As anticipated, several potent inhibitors with increased solubility could be identified. Similar to the substituted phenyl series (Table 1) the SAR regarding



Scheme 4. (a) 5 Mol% [$RuCl_2(PCy_3)_2$(=CHPh)] (Grubbs catalyst), methylene chloride, reflux, 72 h, 80%.

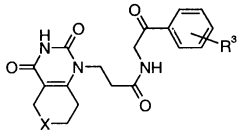
R^3 is relatively broad with selected EC_{50} values being higher than expected (e.g., **27**, **33**).

The most promising piperazine based compounds **24**, **25**, **26**, **28**, **29** and **32** were further investigated in PK studies. Besides various substitutions at the phenyl and piperazine group (R^3 and R^4) different spacer moieties have also been examined (Table 3). It could be shown that the ßAla-Gly type spacer in **2**–**34** proved to be an optimum whereas modification led to a moderate (**35**–**37**, **39**) or drastic loss (**38**, **40**) in activity. Inversion of the ßAla-Gly spacer to Gly-ßAla as in **36** resulted only in a threefold loss of activity. These findings indicate that the spacer is crucial for remaining biological activity. The reason behind this might be a better stabilisation of a favourable conformation of R^2 in an inhibitor-enzyme complex.

Apart from side chain R^2 the (hetero) cyclohexene moiety was also widely varied (Table 4). Substituted cyclohexenones resulted in a drastic loss of potency and as shown in Tables 1 and 2 it could be confirmed that only a 3,6-dihydro-2-thiopyrane subunit (X = S) as in **32** resulted in an improvement of biological activity compared to the cyclohexene core as in **31**. All other replacements including exchange of heteroatoms (e.g., **41**) and variation of the ring size (e.g., **42**, **43**) led to a decrease in potency. This also includes **45** in which the ethylene moiety was introduced as a potential biosteric replacement for sulfur in **32**.[18]

Some of the most potent derivatives were administered intravenously to male Wistar rats in order to determine the efficiency of elimination from the systemic circulation (clearance). Table 5 shows PK-parameters for a number of selected compounds. Medium clearance values were determined for **11**, **20**, **29** and **32** whereas **24**, **25**, **26** and **28** showed high clearances. The determined

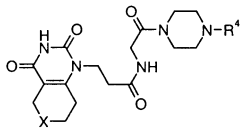
Table 1. Structure–activity relationship for compounds bearing βAla-Gly-phenyl based side chains



Compd	X, R^3	IC_{50} (nM)	EC_{50} (nM)	Sol. (mg/L)
2	C, H	450	n.d.	n.d.
3	C, *p*-OMe	300	n.d.	n.d.
4	S, *p*-OMe	25	80	29
5	S, *p*-Cl	60	500	n.d.
6	S, *p*-$OCHF_2$	30	200	130
7	C, *p*-Br	80	900	10
8	S, *p*-Br	15	70	34
9	S, *m*-Br	70	3500	n.d.
10	S, *p*-*N*-pyrrolidinyl	12	100	15
11	C, $^{\prime}$Ph-$R^{3\prime}$ = 2-naphthyl	25	800	18
12	S, $^{\prime}$Ph-$R^{3\prime}$ = 2-naphthyl	8.5	80	10
13	S, *p*-Ph	20	500	< 1
14	S, *m*-Ph	70	2000	n.d.
15	S, *p*-(O-phenyl)	30	500	10
16	S, *p*-(OCO-phenyl)	30	500	2
17	S, *p*-(4-OMe)-phenyl	15	200	< 1
18	S, *p*-(3-pyridinyl)	12	200	34
19	S, *p*-(4-pyridinyl)	15	100	112
20	S, *p*-pyridazinyl	25	70	305
21	S, *p*-(5-pyrimidinyl)	25	400	n.d.
22	S, *p*-(3-NH_2)-phenyl	20	70	18

n.d.: Not determined.

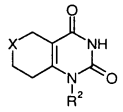
Table 2. Structure–activity relationship for compounds bearing βAla-Gly-piperazine based side chains



Compd	X, R^4	IC_{50} (nM)	EC_{50} (nM)	Sol. (mg/L)
23	C, phenyl	200	n.d.	36
24	S, phenyl	30	100	330
25	S, 4-(MeO)-phenyl	20	70	150
26	S, 2-pyridinyl	35	100	470
27	S, 4-pyridinyl	40	> 1000	110
28	S, 3-(CF_3)-2-pyridinyl	40	100	100
29	S, 4-(CF_3)-2-pyridinyl	20	100	117
30	S, 3-(NH_2)-2-pyridinyl	70	700	n.d.
31	C, 2-pyrimidinyl	90	300	n.d.
32	S, 2-pyrimidinyl	20	100	380
33	S, pyridazinyl	20	400	n.d.
34	S, triazinyl	50	600	n.d.

n.d.: Not determined.

Table 3. Structure–activity relationship for compounds bearing different spacer moieties



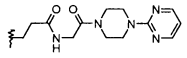
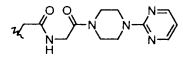
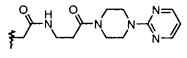
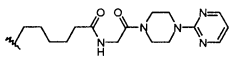
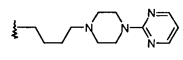
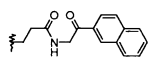
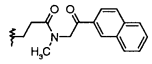
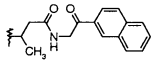
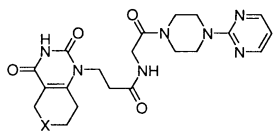
Compds	X	R^2	IC_{50} (nM)
32	S		20
35	S		150
36	S		60
37	S		250
38	C		3000
11	C		25
39	C		400
40	C		> 5000

Table 4. Structure–activity relationship for compounds bearing core variations



Compd	X	IC_{50} (nM)
31	C	90
32	S	20
41	O	800
42	—	3000
43	CH_2-CH_2	900
44	2×(CH=CH_2)	>5000
45	CH=CH	900

Table 5. Pharmacokinetic parameters of selected compounds in male Wistar rats (1 mg/kg iv)

Compd	AUC (mg×h/L)	Cl (L/h×kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)
11	0.60	1.70	0.50	0.7
20	0.61	1.64	0.27	0.3
24	0.23	4.33	1.39	0.4
25	0.16	>4.50	1.01	0.2
26	0.32	3.11	1.19	1.2
28	0.23	4.30	n.c.	n.c.
29	0.45	2.21	0.42	0.3
32	0.52	2.01	0.31	0.2

n.c.: Not calculated.

values for $t_{1/2}$ were relatively short, but compatible with the anticipated application route (iv infusion). Regarding the overall profile **20** and **32** are the most promising candidates and are under further evaluation.

In conclusion, we have discovered and optimised substituted uracil derivatives as a new class of PARP-1 inhibitors. Apart from high potency, compounds with good solubility and medium clearance in rat could be identified.

Acknowledgements

The authors are grateful to Dr. M. Härter, Dr. G. Handke, Dr. A. Jensen and Dr. J. Keldenich for helpful discussions.

References and Notes

1. (a) Ha, H. C.; Snyder, S. H. *Neurobiol. Dis.* **2000**, *7*, 225. (b) Pieper, A. A.; Verma, A.; Zhang, J.; Snyder, S. H. *Trends Pharmacol. Sci.* **1999**, *20*, 171. (c) Chiarugi, A. *Trends Pharmacol. Sci.* **2002**, *23*, 122.
2. (a) Zhang, J.; Dawson, V. L.; Dawson, T. M.; Snyder, S. H. *Science* **1994**, *263*, 687. (b) Lo, E. H.; Bosque-Hamilton, P.; Meng, W. *Stroke* **1998**, *29*, 830.
3. (a) Endres, M.; Wang, Z. Q.; Namura, S.; Waeber, C.; Moskowitz, M. A. *J. Cereb. Blood Flow Metab.* **1997**, *17*, 1143. (b) Takahashi, K.; Pieper, A. A.; Croul, S. E.; Zhang, J.; Snyder, S. H.; Greenberg, J. H. *Brain Res.* **1999**, *829*, 46.
4. (a) Szabo, C.; Dawson, V. L. *Trends Pharmacol. Sci.* **1998**, *19*, 287. (b) Thiemermann, C.; Bowes, J.; Myint, F. P.; Vane, J. R. *Proc. Natl. Acad. Sci.* **1997**, *94*, 679. (c) Plaschke, K.; Kopitz, J.; Weigand, M. A.; Martin, E.; Bardenheuer, H. *J. Neurosci. Lett.* **2000**, *284*, 109. (d) Takahashi, K.; Greenberg, J. H. *NeuroReport* **1999**, *10*, 2017.
5. For a recent review on PARP-1 inhibitors see Larner, A. J. *Expert Opin. Ther. Patents* **2002**, *12*, 481.
6. Costantino, G.; Macchiarulo, A.; Camaioni, E.; Pellicciari, R. *J. Med. Chem.* **2001**, *44*, 3786.
7. Suto, M. J.; Turner, W. R.; Arundel-Suto, C. M.; Werbel, L. M.; Sebolt-Leopold, J. S. *Anti-Cancer Drug Des.* **1991**, *6*, 107.
8. Griffin, R. J.; Srinivasan, S.; Bowman, K.; Calvert, A. H.; Curtin, N. J.; Newell, D. R.; Pemberton, L. C.; Golding, B. T. *J. Med. Chem.* **1998**, *41*, 5247.
9. White, A. W.; Almassy, R.; Calvert, A. H.; Curtin, N. J.; Griffin, R. J.; Hostomsky, Z.; Maegley, K.; Newell, D. R.; Srinivasan, S.; Golding, B. T. *J. Med. Chem.* **2000**, *43*, 4084.
10. (a) Soriano, F. G.; Virág, L.; Jagtap, P.; Szabó, É.; Mabley, J. G.; Liaudet, L.; Marton, A.; Hoyt, D. G.; Murthy, K. G. K.; Salzman, A. L.; Southan, G. J.; Szabó, C. *Nature Med.* **2001**, *7*, 108. (b) Li, J.-H.; Serdyuk, L.; Ferraris, D. V.; Xiao, G.; Tays, K. L.; Kletzly, P. W.; Li, W.; Lautar, S.; Zhang, J.; Kalish, V. *J. Bioorg. Med. Chem. Lett.* **2001**, *11*, 1687.
11. Banasik, M.; Komura, H.; Shimoyama, M.; Ueda, K. *J. Biol. Chem.* **1992**, *267*, 1569.
12. For a review see: Gorbatenko, V. I. *Tetrahedron* **1993**, *49*, 3227.
13. Temple, C., Jr.; Wheeler, G. P.; Elliott, R. D.; Rose, J. D.; Kussner, C. L.; Comber, R. N.; Montgomery, J. A. *J. Med. Chem.* **1982**, *25*, 1045. (b) Stegmann, H. B.; Deuschle, G.; Schuler, P. *J. Chem. Soc., Perkin Trans. 2* **1999**, 547.
14. **36/39**: preparation from **V** and the corresponding amines; **38**: preparation from 4-[4-(2-pyrimidinyl)-1-piperazinyl]butylamine; **40**: preparation from ethyl 3-aminobutanoate.
15. For a leading reference see: Fürstner, A. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 3012.
16. Purified recombinant human PARP-1 (Bac-To-Bac expression system; Life Technologies); Assay volume = 110 μL, 62.5 mM Tris–HCl (pH 7.4), 6.5 mM $MgCl_2$, 0.65 mM dithiothreitol, 100 μg/mL sonicated calf thymus DNA, 1 mg/mL type IIA histone, 60 μM [^{14}C]NAD^+ (2.8 kBq), 50 μg/mL PARP-1, compound to be tested; Procedure: (a) incubation of reaction mix for 10 min at rt, (b) completion by adding 1 mL ice cold 10% (w/v) trichloroacetic acid, (c) transfer of the precipitate onto a filter (Printed Filter Mat A; Wallac), washing with ethanol and drying, d) determination of radioactivity by scintillation counting; see also: Iti, S.; Shizuta, Y.; Hayaishi, O. *J. Biol. Chem.* **1979**, *254*, 3647.
17. 7500 MHEC5-T cells/well (DSM ACC 336; german collection of microorganisms and cell cultures) were seeded on 96 well plates and incubated for 24 h at 37 °C, 5% CO_2. Then 3 mM H_2O_2, 6% Alamar blue and the compound to be tested were added. Cells were incubated for 5 h (37 °C, 5% CO_2). Protection of cells was measured as amount of generated fluorescent dye from Alamar blue. Fluorescence was measured at absorption 560 nm/emission 590 nm; see also: Zhang, J.; Lautar, S.; Huang, S.; Ramsey, C.; Cheung, A.; Li, J.-H. *Biochem. Biophys. Res. Commun.* **2000**, *278*, 590.
18. For a review on bioisosterism see: Patani, G. A.; LaVoie, E. *J. Chem. Rev.* **1996**, *96*, 3147.