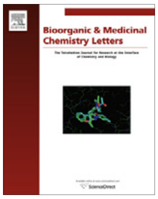
Bioorganic & Medicinal Chemistry Letters 20 (2010) 5165–5169

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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



Discovery of substituted benzyl tetrazoles as histamine H3 receptor antagonists

Adam J. Davenport [a,*], Christopher C. Stimson [a], Massimo Corsi [a], Darshan Vaidya [a], Edward Glenn [a], Timothy D. Jones [a], Sarah Bailey [a], Mark J. Gemkow [b], Ulrike Fritz [b], David J. Hallett [a]

[a] *Evotec (UK) Ltd, 114 Milton Park, Abingdon, Oxfordshire OX14 4SA, United Kingdom*
[b] *Evotec AG, Schnackenburgallee 114, 22525 Hamburg, Germany*

ARTICLE INFO

Article history:
Received 14 May 2010
Revised 2 July 2010
Accepted 3 July 2010

Keywords:
Histamine
H3R
Antagonists
CNS
GPCR
Drug discovery

ABSTRACT

A series of potent and subtype selective H3 receptor antagonists containing a novel tetrazole core and diamine motif is reported. A one-pot multi-component Ugi reaction was utilised to rapidly develop the structure–activity relationships (SAR) of these compounds. Optimisation for liver microsome stability ($t_{1/2}$ >60 min), minimal CYP inhibition (IC_{50} >50 μM) and high cell permeability (Caco-2 P_{app} >20 × 10^{-6} cm/s) identified several compounds with drug-like properties.

© 2010 Elsevier Ltd. All rights reserved.

The histamine H3 receptor (H3R) is primarily located in the central nervous system and exists as one of four known subtypes alongside the H1, H2 and H4 receptors. With marketed therapeutic agents for the H1R and H2R having been highly successful, the more recently discovered H3R[1] and the H4R are attracting significant interest from both academia and industry as potential therapeutic targets.[2,3] Located mainly in the basal ganglia, hippocampus and cortical areas,[4,5] the H3R subtype acts as an inhibitory auto- and hetero-G-protein coupled receptor. Activation of the receptor decreases the release of histamine whereas antagonism (or inverse agonism) increases the release of histamine.[1,6] As a heteroreceptor, the H3R is capable of regulating the release of other important neurotransmitters, such as acetylcholine, dopamine, serotonin and norepinephrine.[7–10] Preclinical data, as well as publicly available clinical data,[11,12] support the potential utility of H3R antagonists in the amelioration of multiple CNS disorders, including ADHD, AD, schizophrenia and narcolepsy.[13–17]

Early H3R antagonists based around the imidazole core[18] of histamine caused inhibition of cytochrome P_{450} enzymes and were probably not progressed into the clinic due to the inherent risk of undesirable drug–drug interactions (DDI's).[19,20] Various laboratories have subsequently developed non-imidazole based H3 receptor antagonists including GSK189254, ABT-288, PF-03654746, MK-0249, JNJ-17216498 and BF2.649 (see Fig. 1 for disclosed structures).[13] These have entered clinical trials for AD, ADHD, schizophrenia, narcolepsy and excessive daytime sleepiness.

Attracted by the potential of the H3R as a drug target, a high throughput screen (HTS) of Evotec's screening collection was performed. This resulted in the identification of compound **1a**; a moderately potent human H3R antagonist with selectivity versus H1,

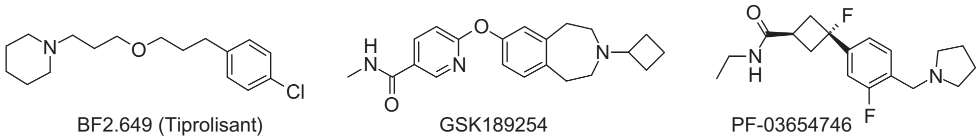
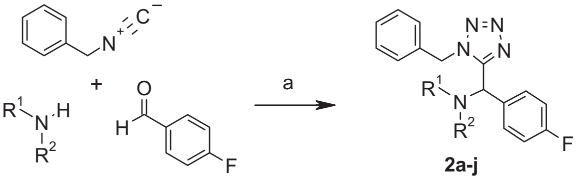

BF2.649 (Tiprolisant)   GSK189254   PF-03654746

Figure 1. Disclosed structures of clinical compounds.

---

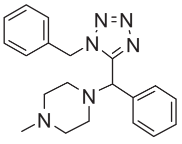
* Corresponding author. Tel.: +44 (0) 1235 838807; fax: +44 (0) 1235 441509.
E-mail address: Adam.Davenport@evotec.com (A.J. Davenport).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.009



Scheme 1. Reagents and condition: (a) TMS azide, MeOH, rt, 18 h. Isolated yields range from 11% to 64%.

H2 and H4 receptors. In this report, the discovery, SAR and development to potent and selective H3R antagonists are described. Early investigations around the novel tetrazole core were undertaken to investigate the SAR around this class of compound.



1a

hH3 IC$_{50}$ = 0.9 μM
rH3 IC$_{50}$ >20 μM
hH1 IC$_{50}$ >20 μM
hH2 IC$_{50}$ >20 μM
hH4 IC$_{50}$ >20 μM

Initial work was carried out varying the cyclic amine to investigate the importance and positioning of the distal basic nitrogen. The multicomponent Ugi reaction[21] was utilised to give rapid access to the desired products in low to high isolated yield as shown in Scheme 1.[22] Reaction of 4-fluorobenzaldehyde with benzylisocyanide, TMS-azide and a range of amine reagents gave a convenient one-pot approach to a series of analogues (**2a–j**) as shown in Table 1.

The first results highlighted the importance of the basic nitrogen where replacement with either carbon or oxygen led to a complete loss of activity (**2a and 2b**). Increasing the number of rotatable bonds in **2c** also showed a marked decrease in potency. Retaining the six-membered piperazine ring and investigating different alkyl piperazine substituents in derivatives **2d–f** gave improved activity; optimal potency of ~25 nM was observed for cyclobutyl and cyclopentyl analogues **2d and 2e**. A similar trend was seen for homopiperazine derivatives **2g–j** demonstrating the optimal positioning and environment of the basic nitrogen atom and revealing the preference of the receptor for small sterically demanding alkyl groups. Potent examples (**2d, 2e** and **2i**) demonstrated >500-fold selectivity for the H3R over H1, H2 and H4 receptor subtypes. The in vitro stabilities of **2d** and **2i** were assessed using human and rat liver microsome assays (HLM and RLM, respectively) indicating rapid clearance ($t_{1/2}$ = <5 min). Metabolite identification studies on **2d** HLM incubate revealed the des-cyclobutyl compound as the major product. Formation of this metabolite can be rationalised via hydroxylation of the cyclobutyl group and subsequent elimination to reveal the secondary amine. Loss of the tertiary amine environment of **2d** had a deleterious effect on H3R potency.

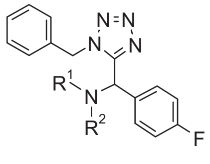
It was rationalised that improved stability could be achieved by blocking the major site of metabolism. This led to the synthesis of substituted piperazine analogues **3a–l** (Table 2).

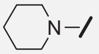
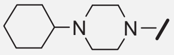
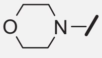
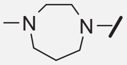
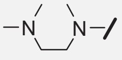
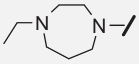
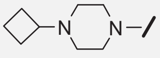
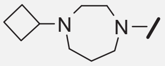
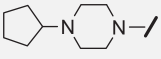
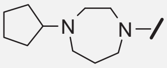
Compounds **3a and 3b** gave excellent human H3R potency (of 2.2 and 3.3 nM, respectively) but shielding around nitrogen did not afford an improvement in metabolic stability. Complete removal of the alkyl ring in **3c** resulted in a marked loss in potency. Further attempts at rigidifying the piperazine ring whilst sterically shielding the distal nitrogen (compounds **3d and 3e**) resulted in up to 20-fold loss of potency with no improvement in stability. Replacement of the cyclobutyl group of **3a** gave a loss in potency (**3f–h**), although a minor increase in stability was observed with the acetylene capped compound **3f**. Moving the distal basic amine to an exocyclic position was accompanied by reduced H3R activity in all cases (**3i–l**), although compound **3j** gave a significant improvement in microsomal stability.

Compound **2i** was selected for further optimisation on the basis of a superior early ADME profile, compared to **2d**. Retaining the active amine of **2i** and modifying the aromatic substituents would allow further SAR to be elucidated and potentially enhance potency. It was anticipated that a global change in molecular shape could also leave the metabolic site less prone to oxidation. Compounds **4a–r** (Table 3) were thus synthesised following the route previously disclosed in Scheme 1.

Table 3 shows the potencies of *ortho* (**4a and 4b**), *meta* (**4c and 4d**) and *para* (**4e–r**) substituted compounds. A significant drop in potency was seen for *ortho* examples **4a and 4b** alongside a smaller

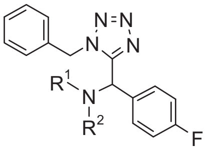
Table 1
Human H3R activities of compounds **2a–j**

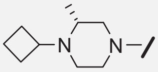
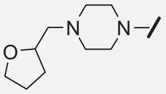
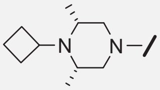
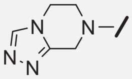
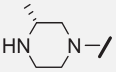
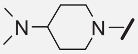
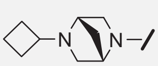
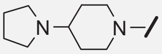
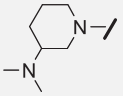
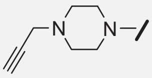
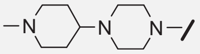


Compound	NR^{1}R^2	hH3R IC$_{50}$[a] (nM)	Compound	NR^{1}R^2	hH3R IC$_{50}$[a] (nM)
2a		20,000 (±0)	**2f**		285 (±160)
2b		20,000 (±0)	**2g**		738 (±297)
2c		2742 (±128)	**2h**		94 (±24.5)
2d		25 (±3.5)	**2i**		47 (±16.7)
2e		31 (±14.3)	**2j**		356 (±88)

See Ref. 22 for complete details of hH3R assay conditions.
[a] Values are arithmetic means of two or more experiments, standard deviation is given in parentheses. Differences of <2-fold should not be considered significant.

Table 2
Human H3R activities for compounds **3a–l**

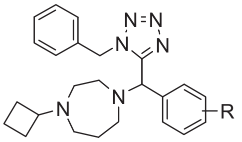


Compound	NR^1R^2	hH3R IC_{50}[a] (nM)	HLM $t_{1/2}$ (min)	Compound	NR^1R^2	hH3R IC_{50}[a] (nM)	HLM $t_{1/2}$ (min)
3a		2.2 (±0.8)	8	**3g**		4894 (±1871)	3
3b		3.4 (±1.7)	3	**3h**		20,000 (±0)	–
3c		1761 (±676)	–	**3i**		3612 (±840)	–
3d		241 (±73)	7	**3j**		2431 (±686)	35
3e		516 (±192)	7	**3k**		2470 (±927)	–
3f		184 (±52)	17	**3l**		14,215 (±8181)	–

See Ref. 22 for complete details of hH3R assay conditions.

[a] Values are arithmetic means of two or more experiments, standard deviation is given in parentheses. Differences of <2-fold should not be considered significant. HLM, human liver microsome.

Table 3
Human H3R activities for compounds **4a–r**



Compound	R substituent	hH3R IC_{50}[a] (nM)	HLM $t_{1/2}$ (min)
4a	2-Cl	475 (±109)	–
4b	2-$OCHF_2$	1891 (±535)	–
4c	3-Cl	64 (±34)	–
4d	3-$OCHF_2$	58 (±2.9)	13
4e	4-H	17 (±3.4)	–
4f	4-^{i}Pr	28 (±0.7)	9
4g	4-CF_3	42 (±20)	–
4h	4-OEt	14 (±9)	12
4i	4-$OCHF_2$	28 (±4)	9
4j	4-OCF_3	51 (±26)	21
4k	4-Cl	34 (±11)	21
4l	4-Br	115 (±26)	–
4m	4-$CONH_2$	19 (±6)	–
4n	4-CONHMe	3.0 (±2.3)	35
4o	4-CONHPh	0.3 (±0.04)	–
4p	4-Pyrrolidin-2-One	21 (±7)	34
4q	4-SO_2Me	1.6 (±0.4)	40
4r	4-SO_2NMe_2	6.8 (±1.2)	–

See Ref. 22 for complete details of hH3R assay conditions.

[a] Values are arithmetic means of two or more experiments, standard deviation is given in parentheses. Differences of <2-fold should not be considered significant. HLM, human liver microsome.

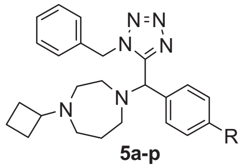
decrease with *meta* substituted examples **4c and 4d**, compared to the *para* analogues **4i** and **4k** that retained activity. A large range of *para* substituted functionalities were tolerated including alkyl (**4f and 4g**), alkoxy (**4h–j**), halo (**4k and 4l**), amide (**4m and 4p**), sulfone (**4q**) and sulfonamide (**4r**) groups. The comparable potencies observed with both electron withdrawing and donating groups suggests that the electron density on the aromatic ring does not significantly influence the binding. Compared to the unsubstituted analogue **4e**, only amide, sulfone and sulfonamide examples **4n–r** gave significantly improved potencies (up to $IC_{50} = 0.3$ nM for **4o**). We inferred that further amino acid residues might be available for additional hydrogen bonding interactions within the receptor binding site, which presented further opportunity for potency optimisation. A modest increase in human microsomal stability was observed with alkoxy substituents (**4h–j**) with half-lives of up to 21 min (**4j**). Gratifyingly, amides **4n** and **4p** as well as sulfone **4q** were more stable still, with half-lives of up to 40 min.

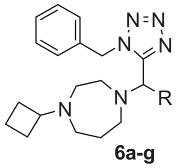
Encouraged by these results, a range of analogues containing *para* substituted heterocycles was synthesised to take advantage of these findings (Table 4).

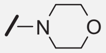
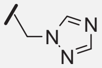
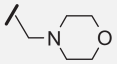
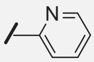
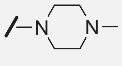
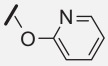
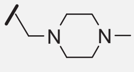
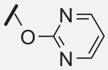
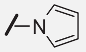
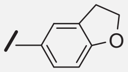
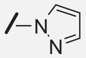
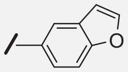
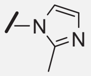
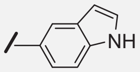
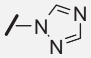
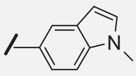
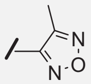
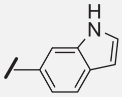
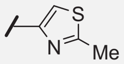
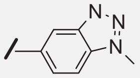
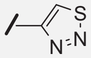
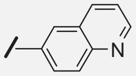
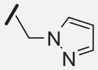
Morpholine and piperazine analogues **5b–d** showed excellent sub-nanomolar potencies, although **5a** was unexpectedly less potent ($IC_{50} = 550$ nM). Compound **5d** had an improved half-life of 74 min and demonstrated the tolerance of the receptor to an additional basic centre.

Five-membered aromatic heterocycles **5e**–**k** were potent (IC_{50} <10 nM), again demonstrating the tolerance for a wide range of functionalities. Unfortunately, microsomal stabilities were generally undesirably low ($t_{1/2}$ = <25 min). The addition of the methylene spacer in **5m** retained potency and gave an increase in half-life

Table 4
Human H3R activities and microsomal stabilities for **5a–p** and **6a–g**


5a-p


6a-g

Compound	R substituent	hH3R IC_{50}[a] (nM)	HLM $t_{1/2}$ (min)	Compound	R substituent	hH3R IC_{50}[a] (nM)	HLM $t_{1/2}$ (min)
5a		551 (±435)	–	**5m**		4.6 (±1.9)	32
5b		0.6 (±0.3)	14	**5n**		5.9 (±0.6)	16
5c		0.5 (±0.002)	23	**5o**		0.9 (±0.2)	–
5d		0.4 (±0.4)	74	**5p**		12 (±7)	13
5e		6.5 (±1.6)	5	**6a**		58.5 (±27.7)	20
5f		1.2 (±0.4)	7	**6b**		41 (±7)	23
5g		0.2 (±0.04)	25	**6c**		119 (±33)	–
5h		1.6 (±0.1)	12	**6d**		98 (±13)	9
5i		9.2 (±1.5)	–	**6e**		32 (±14.4)	10
5j		0.4 (±0.1)	11	**6f**		33 (±3)	27
5k		3.6 (±1.7)	8	**6g**		102 (±7.6)	–
5l		0.7 (±0.2)	11				

See Ref. 22 for complete details of hH3R assay conditions.

[a] Values are arithmetic means of two or more experiments, standard deviation is given in parentheses. Differences of <2-fold should not be considered significant. HLM, human liver microsome.

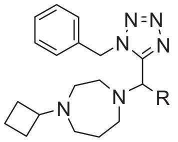
to 32 min (relative to **5h**). The pyridine and pyrimidine substituents in examples **5n–p** also exhibited good potency but offered no improvement in microsomal stability relative to the 5-membered aromatic heterocycles. The introduction of fused heterocycles in **6a–g** reduced potency and provided no improvement in stability. Tetrazole compounds exhibit low species specificity for human over rat H3R, data for representative examples is shown in Table 5, with potent compound **5d** being equipotent at both human and rat H3R.

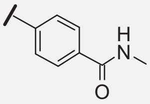
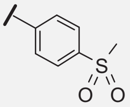
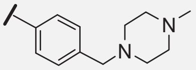
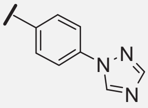
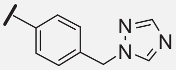
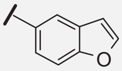
The most promising compounds were profiled in further in vitro assays to assess their cell permeability properties and CYP_{450} inhibition.

As depicted in Table 5, the permeability data obtained from Caco-2 monolayer studies show, with the exception of **5h** and **6b**, that the compounds tested have a low rate of transport across the cell membrane. The efflux ratios of >40 indicated that these compounds are also likely to be substrates for ABC transporters (such as P-glycoprotein). Gratifyingly, Caco-2 data from compounds **5h** and **6b** suggested that discrete structural modifications can circumvent these issues to give highly permeable compounds with minimal efflux. The CYP_{450} inhibition data highlighted that the compounds tested generally have the propensity to inhibit CYP2D6 (IC_{50} = 0.6–1.7 μM) and in some cases (**5h**, **5m** and **6b**) moderate inhibition of CYP3A4 was also observed. Methylsulfone **4q** had a clean CYP_{450} profile, highlighting how varying substituents on the phenyl ring can have a significant effect on the in vitro profile.

In conclusion, we report here the discovery and development of a series of potent and subtype selective histamine H3R antagonists. In vitro testing demonstrated that selected examples have moderate microsomal stability and that structural modifications gave highly permeable compounds with minimal CYP_{450} inhibition. Further optimisation is ongoing to give the desired balance of properties and will be reported in follow-up publications.

Table 5
In vitro data for selected compounds



Compound	R substituent	M_W	TPSA	$C \log P$	hH3R IC$_{50}$[a] (nM)	rH3R IC$_{50}$[a] (nM)	HLM $t_{1/2}$ (min)	Caco-2 A-B[b]	Caco-2 B-A/A-B[b]	CYP2D6 Inhib.[c] (μM)	CYP3A4 Inhib.[c] (μM)
4n		459.6	79.2	2.2	3.0 (±2.3)	12 (±5)	35	0.3	127	1.4	>50
4q		480.6	84.2	1.9	1.6 (±0.4)	17 (±8)	40	0.9	62	>50	>50
5d		514.7	56.6	3.8	0.4 (±0.4)	0.4 (±0.3)	74	0.8	46	1.7	>50
5h		469.6	80.8	3.2	1.6 (±0.1)	9.2 (±3.5)	12	104	2.5	0.7	29
5m		483.6	80.8	3.0	4.6 (±1.9)	17 (±5)	32	0.5	90	0.6	12
6b		442.6	63.2	4.2	41 (±7)	138 (±46)	23	41	0.7	1	2.4

See Ref. 22 for complete details of hH3R assay conditions.

[a] Values are arithmetic means of two or more experiments, standard deviation is given in parentheses. Differences of <2-fold should not be considered significant. HLM, human liver microsome.

[b] Caco-2 units: P_{app} 1×10^{-6} cm/s.

[c] CYP P_{450} inhibition reported as IC$_{50}$ values.

Acknowledgements

The authors thank the ADMET group (notably Anna Pedret-Dunn and Wayne Thomas) for performing ADME studies, Mark Cobb for chemical syntheses and Andreas F. Kahrs, Christian Kirchhoff and the HTS Group for conducting the H3R HTS.

References and notes

1. Arrang, J.-M.; Garbarg, M.; Schwartz, J.-C. *Nature* **1983**, *302*, 832.
2. Celanire, S.; Wijtmans, M.; Talaga, P.; Leurs, R.; de Esch, I. J. P. *Drug Discovery Today* **2005**, *10*, 1613.
3. Thurmond, R. L.; Gelfand, E. W.; Dunford, P. J. *Nat. Rev. Drug Discovery* **2008**, *7*, 41.
4. Drutel, G.; Peitsaro, N.; Karlstedt, K.; Wieland, K.; Smit, M. J.; Timmerman, H.; Panula, P.; Leurs, R. *Mol. Pharmacol.* **2001**, *59*, 1.
5. Martinezmir, M.-I.; Pollard, H.; Moreau, J.; Arrang, J. M.; Ruat, M.; Traiffort, E.; Schwartz, J. C.; Palacios, J. M. *Brain Res.* **1990**, *526*, 322.
6. Morisset, S.; Rouleau, A.; Ligneau, X.; Gbahou, F.; Tardivel-Lacombe, J.; Stark, H.; Schunack, W.; Ganellin, C. R.; Schwartz, J. C.; Arrang, J. M. *Nature* **2000**, *408*, 860.
7. Schlicker, E.; Betz, R.; Göthert, M. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1988**, *337*, 588.
8. Schlicker, E.; Fink, K.; Hinterthaner, M.; Göthert, M. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1980**, *6*, 633.
9. Schlicker, E.; Fink, K.; Detzner, M.; Göthert, M. *J. Neural Transm–General Section* **1993**, *1*, 1.
10. Clapham, J.; Kilpatrick, G. J. *Br. J. Pharmacol.* **1992**, *4*, 919.
11. Lin, J.-S.; Dauvilliers, Y.; Arnulf, I.; Bastuji, H.; Anaclet, C.; Parmentier, R.; Kocher, L.; Yanagisawa, M.; Lehert, P.; Ligneau, X.; Perrin, D.; Robert, P.; Roux, M.; Lecomte, J.-M.; Schwartz, J.-C. *Neurobiol. Dis.* **2008**, *30*, 74.
12. http://www.gsk.com/investors/presentations/2007/neurosciences-seminar-dec07/jackie-hunter.pdf.
13. Gemkow, M. J.; Davenport, A. J.; Harich, S.; Ellenbroek, B. A.; Cesura, A.; Hallett, D. *Drug Discovery Today* **2009**, *14*, 509.
14. Passani, M. B.; Lin, J.-S.; Hancock, A.; Crochet, S.; Blandina, P. *Trends Pharmacol. Sci.* **2004**, *25*, 618.
15. Wijtmans, M.; Leurs, R.; de Esch, I. *Expert Opin. Invest. Drugs* **2007**, *7*, 967.
16. Bongers, G.; Bakker, R. A.; Leurs, R. *Biochem. Pharmacol.* **2007**, *8*, 1195.
17. Hancock, A. A. *Biochem. Pharmacol.* **2006**, *8*, 1103.
18. Cowart, M.; Altenbach, R.; Black, L.; Faghih, R.; Zhao, C.; Hancock, A. A. *Mini Rev. Med. Chem.* **2004**, *9*, 979.
19. Boxenbaum, H. *J. Pharm. Pharm. Sci.* **1999**, *2*, 47.
20. Lin, J. H.; Lu, A. Y. H. *Clin. Pharmacokinet.* **1998**, *35*, 361.
21. Ugi, I.; Meyer, R.; Fetzer, U.; Steinbruckner, C. *Angew. Chem.* **1959**, *71*, 386.
22. For example, the synthesis of compound **2i** is described in Ref. 23.
23. Davenport, A. J.; Hallett, D. J.; Stimson, C. C.; Corsi, M.; Gemkow, M. WO20095394, 2009.