Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1307–1311
Bioorganic & Medicinal Chemistry Letters

# Synthesis and biological evaluation of fenobam analogs as mGlu5 receptor antagonists

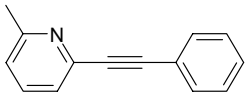
Georg Jaeschke,* Richard Porter, Bernd Büttelmann, Simona M. Ceccarelli, Wolfgang Guba, Bernd Kuhn, Sabine Kolczewski, Jörg Huwyler, Vincent Mutel, Jens-Uwe Peters, Theresa Ballard, Eric Prinssen, Eric Vieira, Jürgen Wichmann and Will Spooren

*Pharmaceutical Division, Discovery Research, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland*

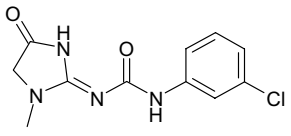
Received 17 October 2006; revised 1 December 2006; accepted 3 December 2006
Available online 15 December 2006

**Abstract**—Optimization of affinity and microsomal stability led to identification of the potent, metabolically stable fenobam analog **4l**. Robust in vivo efficacy of **4l** was demonstrated in four different models of anxiety. Additionally, a ligand based pharmacophore alignment of fenobam and MPEP is proposed.


L-Glutamate, the major excitatory amino acid neurotransmitter in the central nervous system, binds to and activates several classes of receptors, which are divided into two groups termed ionotropic (iGlu) and metabotropic glutamate receptors (mGlu).[1] The mGlu receptors are classified based on their homology, pharmacology, and second messengers in three groups.[2] The mGlu5 receptor belongs to group I mGlu receptors, which are coupled to phospholipase C leading to the activation of phosphoinositide (PI) hydrolysis and elevation of $Ca^{2+}$ levels. The high expression of mGlu5 receptor in the limbic areas of the brain suggests a potential role of this receptor in psychiatric disorders, such as anxiety.



**1**



**2**

MPEP (2-methyl-6-(phenylethynyl)-pyridine) **1** has been reported to be an mGlu5 receptor antagonist and acts via an allosteric binding site located in the transmembrane domain.[3] MPEP is active in a wide range of preclinical anxiety models such as the stress-induced hyperthermia, Vogel conflict and plus maze test.[4]

We have serendipitously discovered in a screening campaign that fenobam [*N*-(3-chlorophenyl)-*N*′-(4,5-dihydro-1-methyl-4-oxo-1*H*-imidazole-2-yl)urea] **2** is a potent, subtype-selective, and non-competitive mGlu5 receptor antagonist.[5] Fenobam entered clinical trials for anxiety in the late seventies and proved to be similarly active as benzodiazepines in a double blind placebo-controlled clinical trial, but did not show the same liabilities such as ethanol interaction and sedation.[6]

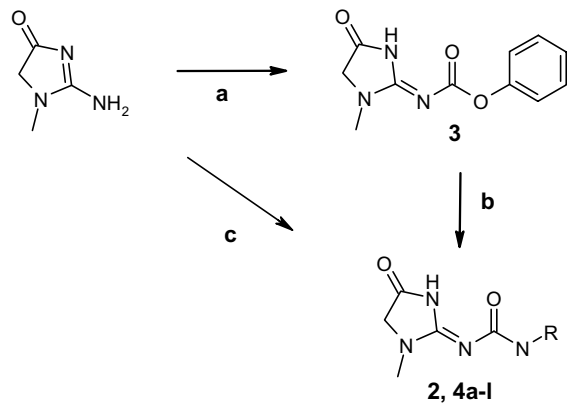
With studies using point mutated mGlu5 receptors and 3-D receptor pharmacophore-based modeling, we have demonstrated recently that the two structurally diverse allosteric antagonists MPEP and fenobam have similar contact sites on the mGlu5 receptor binding crevice.[7]

In this paper, we would like to report a SAR of fenobam analogs[8] and to propose a ligand based pharmacophore alignment of fenobam and MPEP. In addition, we describe the biological evaluation of fenobam analog **4l** with improved metabolic stability.

To establish a SAR, we decided to vary the 3-chlorophenyl substituent of fenobam. Synthesis was accomplished by either direct condensation of creatinin with the corresponding aniline in the presence of CDI or via a stepwise

*Keywords*: mGluR5; Fenobam; Anxiety; MPEP; Pharmacophore.
* Corresponding author. Tel.: +41 61 68 82034; fax: +41 61 68 88714; e-mail: georg.jaeschke@roche.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.12.033



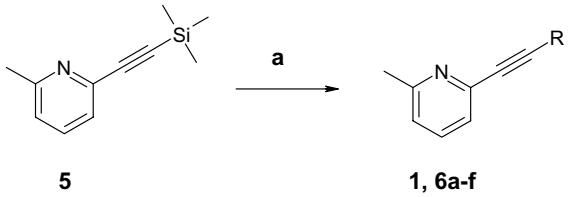

**Scheme 1.** Synthesis of fenobam analogs **2**, **4a**–**l**. Reagents and conditions: (a) phenylchloroformate, THF, reflux, 17%; (b) aniline, DMF, 50 °C, 30–70%; (c) aniline, CDI, DMF, 90 °C or reflux, 20–65%, 1% for **4b**.

procedure using the phenoxycarbamate intermediate **3** (Scheme 1).

To allow a direct comparison with the SAR of MPEP, we also synthesized a representative subset of MPEP analogs varying the phenyl substituent via Sonogashira coupling reaction using intermediate **5**[9] (Scheme 2).

The fenobam analogs show a steep structure–activity relationship. The 3-chloro substituent is of crucial importance and the unsubstituted phenyl analog is significantly less active. Shifting the chloro substituent to the ortho- and para-position also leads to a drop in affinity. The 2- and 4-pyridine derivatives maintain some activity, whereas the 3-pyridine derivative is inactive (Table 1). Hepatic stability (determined in vitro using rat and human liver microsomes) is low for fenobam, an observation that can be rationalized by assuming hydroxylation of the para-position on the phenyl.[10] The more polar pyridine derivatives **4d**–**f** show a higher microsomal stability (Table 1).

In the MPEP series modifications of the phenyl ring are much better tolerated at the mGlu5 receptor compared to fenobam analogs. In particular, the influence of the 3-chloro substituent is of lower importance than for fenobam and a chloro substituent in the 2-position is also tolerated (Table 2). Nevertheless, the SAR of the MPEP derivatives shows some qualitative overlap with fenobam analogs as, for example, the 3-chloro derivatives are most potent whereas the 4-chloro derivatives




**Scheme 2.** Synthesis of MPEP derivatives **1**, **6a**–**f**. Reagents and conditions: (a) ArI, CuI, $Bu_4NF$; $Pd(PPh_3)Cl_2$, $PPh_3$, triethylamine, THF, 50 °C, 36–80%.

**Table 1.** Binding affinity and functional activity of fenobam analogs **2**, **4a**–**f**

| Compound | R | 3H-MPEP (nM) | FLIPR (nM) | CL (r/h) (μl/min/mg prot) |
|---|---|---|---|---|
| **4a** | Ph | 1360 | 3300 | — |
| **4b** | 2-Cl–Ph | 2520 | 6800 | — |
| **2** | 3-Cl–Ph | 61 | 38 | 44/100 |
| **4c** | 4-Cl–Ph | >5000 | >5000 | — |
| **4d** | 2-Py | 587 | 1100 | 22/47 |
| **4e** | 3-Py | >5000 | >5000 | 5/20 |
| **4f** | 4-Py | 1120 | >5000 | 9/11 |

**Table 2.** Binding affinity and functional activity of MPEP analogs **1**, **6a**–**f**

| Compound | R | 3H-MPEP (nM) | FLIPR (nM) |
|---|---|---|---|
| **1** | Ph | 4 | 29 |
| **6a** | 2-Cl–Ph | 5 | 15 |
| **6b** | 3-Cl–Ph | 3 | 7 |
| **6c** | 4-Cl–Ph | 286 | 1020 |
| **6d** | 2-Py | 39 | 400 |
| **6e** | 3-Py | 10 | 66 |
| **6f** | 4-Py | 26 | 213 |

are least potent in both series. Substitution of the phenyl ring by a pyridine in the MPEP series leads only to a moderate drop in affinity, which is less pronounced than substitution of the 3-Cl-phenyl ring by a pyridine in the fenobam series. We believe that the similarities in the SAR of fenobam and MPEP support the hypothesis that the phenyl ring of MPEP and the 3-chloro phenyl substituent of fenobam occupy a similar position in the allosteric mGlu5 binding site. A ligand based pharmacophore model supporting this hypothesis is proposed further below (see modeling section).

As mentioned above, a substituent in the meta-position clearly increases affinity in the fenobam series. We therefore investigated the influence of a chloro- or methyl-substituent in combination with ortho- or para-pyridine moieties (Table 3). Interestingly, the influence of the meta substituents is less pronounced than for the phenyl substituents, but in case of the ortho pyridine derivatives **4g** and **4i** compounds with improved potency were obtained. Microsomal clearance is medium to high for these compounds in both rat and human, and is higher than for the respective para pyridine derivatives **4h** and **4j**. This observation is again in line with a potential hydroxylation of the para-position for the ortho pyridine derivatives. To prove the theory of oxidative

**Table 3.** Binding affinity, functional activity, and microsomal stability of fenobam analogs **4g**–**l**

| Compound | R | 3H-MPEP (nM) | FLIPR (nM) | CL (r/h) (μl/min/mg prot) |
|---|---|---|---|---|
| **4g** | 3-Cl-2-Py | 166 | — | 53/42 |
| **4h** | 3-Cl-4-Py | 663 | — | 31/15 |
| **4i** | 3-Me-2-Py | 320 | 681 | 37/29 |
| **4j** | 3-Me-4-Py | 2670 | 1600 | 14/2 |
| **4k** | 3-Thienyl | 700 | 4016 | –/– |
| **4l** | 5-Cl-3-thienyl[11] | 78 | 434 | 27/55 |

metabolism, we replaced the 2-chloro phenyl substituent of fenobam with the isosteric 3-thienyl substituent leading to the fenobam derivative **4k**. We hypothesized that the moderate potency of **4k** could be improved by introduction of an additional chloro substituent in the 5-position leading to **4l**. Indeed **4l** not only retains reasonable potency but also shows improved metabolic stability compared with fenobam.[11]

Therefore, we profiled **4l** in additional models of anxiety.

We evaluated **4l** in four models for assessing anxiolytic-like activity. In stress-induced hyperthermia, exaggerated responses of the autonomic nervous system to stress are measured using body temperature measurements in mice.[4b] Compound **4l** (3, 10, and 30 mg/kg, po) significantly reduced stress-induced hyperthermia at 10 and 30 mg/kg, while having no effect on the baseline temperature (Fig. 1).

Compound **4l** was evaluated in the Vogel conflict test,[13,4d] in which drinking is suppressed in water-restricted rats by a brief electrical shock every second of cumulative drinking time. Compound **4l**, after an oral dose of 30 mg/kg po, but not 3 or 10 mg/kg po, significantly increased drinking time, consistent with an anxiolytic-like profile (Fig. 2).

In the conditioned emotional response (CER) test[4d] in rats, **4l** significantly and dose-dependently reversed the suppression of lever pressing by stimuli previously associated with foot shock, at 3, 10, and 30 mg/kg po (Fig. 3A). There was no significant effect of treatment on the total number of lever presses made during the 1 h session (Fig. 3B) suggesting that behavioral disruption did not occur at this dose–range (although there was a tendency for a reduction at 30 mg/kg po).

In the conflict test[4d] in rats, **4l** significantly and dose-dependently reversed the suppression of lever pressing associated with foot shock at 3, 10, and 30 mg/kg po (Fig. 4A). There was no significant effect of treatment on the lever pressing during the unpunished periods (Fig. 4B) suggesting that behavioral disruption did not occur at this dose–range.

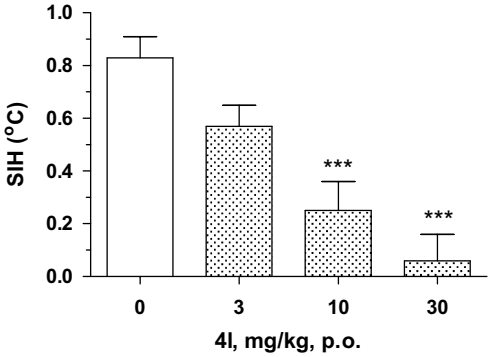



**Figure 1.** Stress-induced hyperthermia (SIH). Data are means and SEM; statistics: ***$p < 0.001$ versus vehicle (Dunnet, two-tailed).

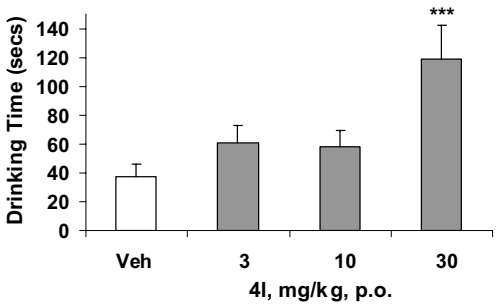



**Figure 2.** Drinking times (sec) in the Vogel conflict test (punished drinking) as a measure of anxiolytic-like response. The drinking times after oral doses of 3, 10, and 30 mg/kg of **4l** (pretreatment time = 60 min) are compared with vehicle. Data are means and SEM; statistics: ***$p < 0.001$ versus vehicle (Mann–Whitney $U$ test, one-tailed).

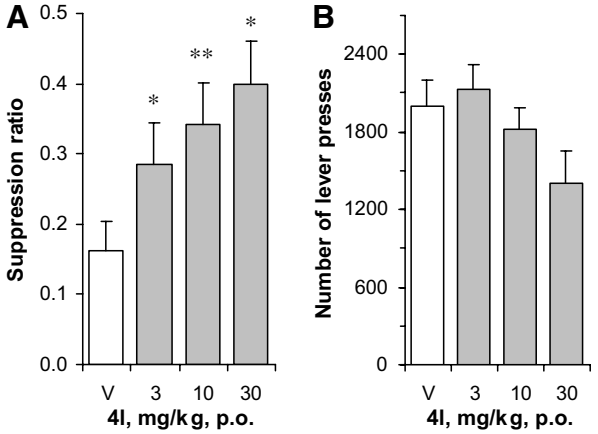



**Figure 3.** (A) Suppression ratio in the CER test as a measure of anxiety; (B) number of lever presses during the 60-min session as a measure of non-specific effects. Data are expressed as means ± SEM. Statistics: *$p < 0.05$, **$p < 0.01$ versus vehicle (A, Wilcoxon rank sum test; B, paired $t$ test with Bonferroni correction).

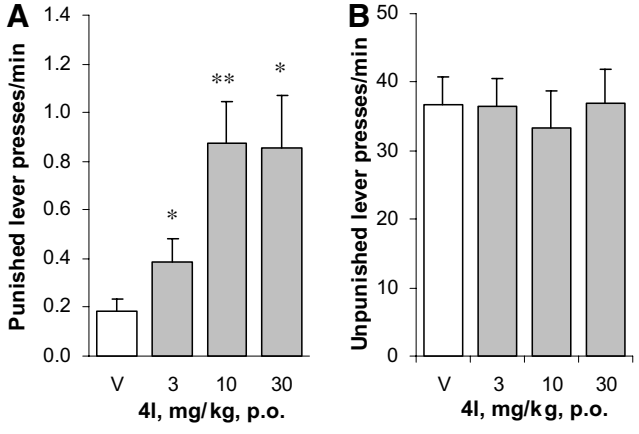



**Figure 4.** (A) Punished lever presses per minute in the conflict test as a measure of anxiety; (B) unpunished lever presses per minute as a measure of non-specific effects. Data are expressed as means ± SEM. Statistics: *$p < 0.05$, **$p < 0.01$ versus vehicle (paired $t$ test with Bonferroni correction).

In conclusion, robust efficacy of **4l** in four different models of anxiety was seen.

Fenobam can exist in two tautomeric forms, depicted in Figure 5, maintaining the energetically favorable C=O bond. To better understand the binding mode to the mGlu5 receptor, we calculated the relative stabilities of the two tautomers using quantum chemical methods.

Geometry optimization at the AM1 level of low-energy conformers of the two tautomers, followed by single-point energy calculation with the B3LYP/cc-pVDZ method, revealed that fenobam with the imino-imidazolidinone motif is substantially more stable than the corresponding amino-dihydroimidazolone tautomer ($\Delta E$ = 19.1 kcal/mol in the gas phase, $\Delta E$ = 10.3 kcal/mol in a PCM continuum solvent description of water).[13] The large preference for the right tautomer in Figure 5 can be understood by the energetically favorable formation of a 6-membered ring through an intramolecular hydrogen bond.

The pharmacophore-guided superposition of MPEP and fenobam is illustrated in Figure 6. Both the central ethynyl and amide linkers of MPEP and fenobam, respectively, are spacers for correctly positioning the benzene rings and the corresponding pyridine or imidazolidinone moieties. As noted in a homology modeling study by Malherbe et al.[7] the common determinants of the MPEP and fenobam binding pockets are hydrophobic interactions with the (chloro)benzene ring and hydrogen bonds with the polar pyridine and imidazolidinone substituents. The pyridine nitrogen of MPEP overlaps with the doubly bonded nitrogen of the most stable fenobam tautomer, which leaves the carbonyl oxygen of the imidazolidinone ring free for an additional H-bond with S3.39.

Since the described variations of R groups modulate the formation and the strength of this additional H-bond, the SAR differences between the MPEP and fenobam series can be rationalized. The torsional twist induced by 2-Cl or the additional steric bulk introduced by 4-Cl is very likely to disrupt this H-bond. Similarly, replacements of the 3-Cl-phenyl ring with pyridine substitutions are not tolerated within the fenobam as opposed to the MPEP series, since fenobam has less translational and rotational degrees of freedom than MPEP due to this additional directed hydrogen bond. Therefore, the introduction of an additional H-bond acceptor via pyridine substitutions cannot be properly accommodated by reorientations within the binding pocket.

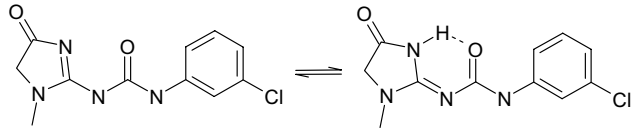



**Figure 5.** Representation of two tautomeric forms of fenobam.

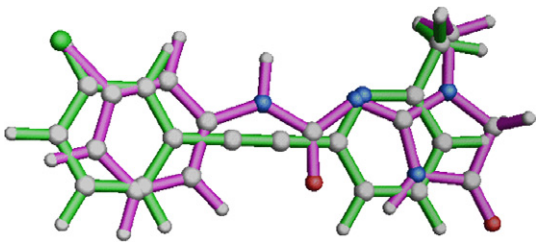


**Figure 6.** Pharmacophore-guided superposition of MPEP (green) and fenobam (purple). Both hydrophobic moieties at the left side of the central spacers and hydrogen bond acceptors of the polar groups at the right side are optimally overlayed.

In conclusion, we could establish in the present report a ligand based pharmacophore alignment between MPEP and fenobam, and characterize in vitro as well as in vivo several fenobam analogs including analog **4l** with improved metabolic stability. In view of their anxiolytic properties in different animal models, the presented fenobam analog **4l** could be of potential interest for the treatment of psychiatric disorders.

## Acknowledgments

The excellent technical assistance of Daniel Rueher, Antonio Ricci, Francoise Kahn, Severine Weil-Bandinelli, and Sean Durkin is gratefully acknowledged.

## References and notes

1. (a) Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. *Pharmacol. Rev.* **1999**, *51*, 7; (b) Pin, J.; De Colle, C.; Bessis, A.; Acher, F. *Eur. J. Pharmacol.* **1999**, *375*, 277; (c) O'Hara, L. J.; Sheppard, P. O.; Thogersen, H.; Venezia, D.; Haldema, B. A.; MacGrane, V.; Houamed, K. M.; Thomsen, C.; Gilbert, E. R.; Mulvihill, E. R. *Neuron* **1993**, *11*, 41.
2. (a) Conn, J. P.; Pin, J. P. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205; (b) Knöpfel, T.; Kuhn, R.; Allgeier, H. *J. Med. Chem.* **1995**, *38*, 1417.
3. Pagano, A.; Ruegg, D.; Litschig, S.; Stoehr, N.; Stierlin, C.; Heinrich, M.; Floersheim, P.; Prezeau, L.; Carroll, F.; Pin, J.-P.; Cambria, A.; Vranesic, I.; Flor, P. J.; Gasparini, F.; Kuhn, R. *J. Biol. Chem.* **2000**, *275*, 33750.
4. (a) Gasparini, F.; Lingenhohl, K.; Stoehr, N.; Flor, P. J.; Heinrich, M.; Vranesic, I.; Biollaz, M.; Allgeier, H.; Heckendorn, R.; Urwyler, S.; Varney, M. A.; Johnson, E. C.; Hess, S. D.; Rao, S. P.; Sacaan, A. I.; Santori, E. M.; Velicelebi, G.; Kuhn, R. *Neuropharmacology* **1999**, *38*, 1493; (b) Spooren, W. P. J. M.; Gasparini, F.; Salt, T. E.; Kuhn, R. *Trends Pharmacol. Sci.* **2001**, *22*, 331; (c) Spooren, W.; Vassout, A.; Neijt, H. C.; Kuhn, R.; Gasparini, F.; Roux, S.; Porsolt, R. D.; Gentsch, C. *J. Pharmacol. Exp. Thera.* **2000**, *295*, 1267; (d) Ballard, T. M.; Woolley, M. L.; Prinssen, E.; Huwyler, J.; Porter, R.; Spooren, W. *Psychopharmacology* **2005**, *179*, 218.
5. Porter, R. H. P.; Jaeschke, G.; Spooren, W.; Ballard, T.; Buettelmann, B.; Kolczewski, S.; Peters, J.-U.; Prinssen, E.; Wichmann, J.; Vieira, E.; Muehlemann, A.; Gatti, S.; Mutel, V.; Malherbe, P. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 711.
6. Pecknold, J. C.; McClure, D. J.; Appeltauer, L.; Wrzesinski, L.; Allan, T. *J. Clin. Psychopharmacol.* **1982**, *2*, 129.
7. Malherbe, P.; Kratochwil, N.; Muhlemann, A.; Zenner, M.-T.; Fischer, C.; Stahl, M.; Gerber, P. R.; Jaeschke, G.; Porter, R. H. P. *J. Neurochem.* **2006**, *98*, 601.

8. See also a recent publication describing the SAR of fenobam analogs 6: Wallberg, A.; Nilsson, K.; Oesterlund, K.; Peterson, A.; Elg, S.; Raboisson, P.; Bauer, U.; Hammerland, L. G.; Mattsson, J. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1142.
9. Alagille, D.; Baldwin, R. M.; Roth, B. L.; Wroblewski, J. T.; Grajkowska, E.; Tamagnan, G. D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 945; Examples 6b, 6d, 6e, and 6f were described previously in the following references: (a) Alagille, D.; Baldwin, R. M.; Roth, B. L.; Wroblewski, J. T.; Grajkowska, E.; Tamagnan, G. D. *Bioorg. Med. Chem.* **2005**, *13*, 197; (b) Allgeier, H.; Auberson, Y.; Biollaz, M.; Cosford, N. D.; Gasparini, F.; Heckendorn, R.; Johnson, E. C.; Kuhn, R.; Varney, M. A.; Velicelebi, G. *PCT Int. Appl.*, 1999, 48pp, WO 9902497.
10. Wu, W. N.; McKown, L. A.; O'Neill, P. J. *J. Pharm. Sci.* **1995**, *84*, 185.
11. (a) For the first description of 4l (=1-(5-Chloro-3-thienyl)-3-(1-methyl-4-oxo-2-imidazolin-2-yl)urea), see: Hunkeler, W.; Kyburz, E.; *Eur. Pat. Appl.*, 1980, 21pp, EP 16371; (b) Bare, T. M. *Eur. Pat. Appl.*, 1980, 28pp, EP79-302921.
12. Vogel, J. R.; Beer, B.; Clody, D. E. *Psychopharmacologia* **1971**, *21*, 1.
13. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, Jr., J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Gaussian, Inc., Pittsburgh PA, 1998.