RESEARCH ARTICLE

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 Cite this: *Med. Chem. Commun.*,
 2018, 9, 789

The semi-synthesis, biological evaluation and docking analysis of the oxime, hydrazine and hydrazide derivatives of platensimycin†

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 Received 11th February 2018,
 Accepted 1st March 2018

DOI: 10.1039/c8md00081f

rsc.li/medchemcomm

A dozen oxime, hydrazine and hydrazide derivatives of platensimycin (PTM) analogues were synthesized, some of which showed strong antibacterial activities and were shown to be stable under the bioassay conditions. Docking analysis revealed that they have certain new interactions with β -ketoacyl-[acyl carrier protein] synthase II (FabF), suggesting that Schiff base formation on its terpene scaffold is an effective strategy to diversify PTM structure.

Introduction

The carbon–carbon bond forming decarboxylating Claisen condensation in the biosynthesis of fatty acids is catalyzed by fatty acid synthases (FASs). FabH or FabB/F in bacteria type II FASs, are responsible for the initiation or elongation of fatty acid biosynthesis, using acetyl-CoA or acyl carrier protein (ACP) thioesters as substrates, respectively. They share a common thiolase fold, but contain different catalytic triads of Cys–His–Asn or Cys–His–His.¹ These condensation enzymes are intensively investigated as promising antibacterial drug targets, because they are conserved in many multi-drug resistant pathogens, while their human homologues are multi-component type I FASs, structurally distinct from bacterial type II FASs.² Several natural products or their synthetic derivatives with potent antibacterial activities were identified, such as cerulenin, thiolactomycin, C-75, phomallenic acids, fasamycins, as well platensimycin (PTM, 1) and platencin (PTN, 2).^{3–6}

Both PTM and PTN consist of a 3-amino-2,4-dihydroxy benzoate (ADHBA) moiety and an unprecedented tetracyclic or tricyclic terpene cage, connected through a propionamide chain (Scheme 1).^{5–8} The important structure–activity relationship (SAR) of PTM has been revealed through synthesis or isolation of their congeners from fermentation: even minor perturbation of the ADHBA moiety would lead to the complete loss of antibacterial activity, while moderate change of the terpene cage often yield active analogues, including carboplatensimycin (3), adamantaplatensimycin (4), 7-phenyl- and 11-methyl 7-phenylplatensimycin (5 and 6).^{9–12} Singh and co-workers prepared several enone-modified PTM analogues, and the co-crystallization study of some active compounds revealed critical interaction of the modified terpene scaffold with *E. coli* (ec)FabF(C163Q).¹³ For example, the enone carbonyl oxygen of PTM, 6,7-dihydroplatensimycin (7) and the

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8md00081f

Scheme 1 The structures of platensimycin and platencin (A) and some selected platensimycin derivatives (B).

7-phenyl dihydroplatensimycin could form a hydrogen bond with Ala309. In addition, the enone olefin on the terpene scaffold is also important to maintain the active and stable conformation of PTM, because the 6,7-dihydroplatensimycin 7, obtained through quantitative palladium-catalyzed hydrogenation, could equilibrate to its cyclic enamino-amido product 8 under acid conditions.^{14,15} Moreover, reduction of PTM by sodium borohydride resulted in the mixture of di- and tetra-hydro derivatives 9–12, which could not be easily isolated and characterized due to their instability.¹⁴

Semi-synthesis is an effective approach to quickly assemble a series of PTM analogues. We have recently reported the facile synthesis of dozens of PTM sulfur analogues through a stereoselective biomimetic sulfur Michael addition approach, as well as its halogen-substituted aminobenzoic acid analogues.^{16,17} To our knowledge, there is no reported simple method to diversify PTM structure on the enone carbonyl oxygen, while maintaining its active conformation. In this study, we report a straightforward approach to modify PTM through Schiff base formation on the terpene scaffold, taking advantage of the large amount of PTM available through fermentation. Several semi-synthesized PTM analogues have potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and were shown to be stable under the bioassay conditions. The docking study revealed that the most active PTM analogues adopted the similar structure conformation with PTM, which might bind to ecFabF(C163Q) effectively.

Results and discussion

Several Schiff base derivatives have been designed to interact with *E. coli* FabH, with strong antibacterial activities towards both Gram-positive and Gram-negative pathogens.^{18,19} Semicarbazones and thiosemicarbazones have also been shown to have excellent antiviral, anti-infective and antineoplastic activities, which were regarded as privileged structures to bind to metal ions in cells.²⁰ We hypothesized that replacing C=O bond with C=N bond in the cyclohexenone of PTM might mimic its original conformation, thus retaining the similar binding modes with PTM in the active site pocket of FabF. In addition, the straightforward semi-synthesis of these PTM analogues might be a very efficient way to diversify PTM scaffold.

First, PTM oxime derivative 13 was quickly obtained through the reaction between hydroxylamine and PTM, with an isolated yield of 83% (Scheme 2A). The structure of 13 was confirmed by extensive 1D and 2D NMR spectroscopy (Fig. S18†). Interestingly, Michael-type addition of hydroxylamine towards the enone occurred, and similar to the previous reported PTM derivatives, only *si*-face product was obtained.^{14,17} Compound 14 was generated from the β -elimination of 13, in the presence of 1.5 eq. of NaOH in MeOH, with a yield of 42%. Next twelve different PTM hydrazine or hydrazone derivatives were synthesized with moderate yields, in which the original keto group was replaced with an hydrazine or hydrazone group (Scheme 2B). In brief, treat-

Scheme 2 The synthesis of PTM analogues. (A) The reaction of hydroxylamine with PTM. 1) Hydroxylamine hydrochloride (5.0 eq.), MeOH, r.t., 24 h; 2) NaOH (1.5 eq.), MeOH, r.t., 48 h. (B) Scope of the PTM derivative formation using alkoxyamine, hydrazine, semicarbazide, thiosemicarbazide and hydrazides. Alkoxyamines or hydrazine derivatives (10.0 eq.), catalytic amount acetic acid, ethanol alcohol, reflux for 24–96 h.

ment of PTM using 10 eq. of alkoxyamines, hydrazine or hydrazide derivatives in ethanol, with catalytic amount acetic acid, facilitated the smooth synthesis of these derivatives. The PTM oxime isomers 15 and 16 were prepared with moderate yields by the reaction of PTM with methoxyamine or ethoxyamine. We have not observed any Michael adducts under the used conditions, probably due to the less electrophilic properties of alkoxyamines. To generate the hydrazine derivative 17 and other hydrazide derivatives 18–26, a diverse set of hydrazine, semicarbazide, thiosemicarbazide and hydrazides were used.

All of the synthesized PTM analogues were tested against *S. aureus* ATCC 29213, several methicillin-sensitive *S. aureus* (MSSA) and MRSA strains, as well as *Klebsiella pneumoniae* and *Escherichia coli* isolated from local hospitals, using the standard agar dilution method with PTM and linezolid as positive controls (Table 1).^{16,21} None of the newly synthesized PTM analogues showed activity against the tested Gram-negative pathogens, including *K. pneumoniae* and *E. coli*, with their minimum inhibitory concentrations (MICs) >64 $\mu\text{g mL}^{-1}$, probably due to the drug efflux mechanisms as observed in PTM.⁵ In contrast, most PTM analogues were active against *S. aureus*, with MICs ranging from 1–64 $\mu\text{g mL}^{-1}$, except the oxime isomers 15 and 16, with MIC > 64 $\mu\text{g mL}^{-1}$. Among them, compound 17 showed the strongest activity, with MICs of 1–2 $\mu\text{g mL}^{-1}$ against all the tested *S. aureus* strains, only about 2–4 fold less potent than PTM. Concerning the stability of these analogues under the bioassay conditions, the most active compound 17, and four other compounds 15, 21, 23 and 25, were tested for their stability in the previous assay conditions. All the tested compounds were stable under the assay condition after overnight incubation (Fig. S19†).

Table 1 The antibacterial activities of PTM analogues against *S. aureus* strains, in comparison to the newly-marketed antibiotic linezolid, as well as PTM and PTN

Tested compounds	MIC ($\mu\text{g mL}^{-1}$)		
	<i>S. aureus</i> ATCC 29213	MSSAs	MRSAs
PTM	0.5	0.5	1
PTN	1	1	1
Linezolid	1	1	1
7	2	2	2
9–12	>64	>64	>64
13	16	16	16
14	32	16	32
15	>64	>64	>64
16	>64	>64	>64
17	2	1	2
18	16	8	16
19	64	16	64
20	64	64	64
21	32	16	32
22	32	16	16–32
23	32	32	64
24	16	16	16
25	8	8	4
26	64	16	64

Binding of PTM to ecFabF(C163Q) would strongly mimic the interaction of PTM towards its target FabF in the antibacterial assay.^{5,15} In order to understand the drastic activity difference among our synthesized PTM analogues, docking studies were performed using Molecular Operating Environment (MOE) platform, with the structure of ecFabF(C163Q) co-crystallized with PTM as the template (PDB ID, 2GFX).²² The docking parameters were obtained when the obtained docking conformation of PTM can reproduce its binding towards ecFabF(C163Q) based on the crystal structure. Then PTM analogues 15, 17 and 25, with no, strong or moderate antibacterial activities, were docked into ecFabF(C163Q) to analyse their interactions with FabF (Fig. 1).

After docking, a pool of 100 optimal conformations was generated for each compound. The most reasonable conformation was selected based on the following two criteria: (1) the ADHBA moiety of the docking compounds would have the similar binding mode with PTM in the FabF active site; (2) the top-scoring conformation was selected for further analysis according the score function in MOE. Our docking results revealed that the terpene cage of compounds 15, 17 and 25 could have some interactions with amino acid residues periphery to the FabF active site (Fig. 1b–d). Compounds 17 and 25 formed a new interaction with Arg206, which correlated well with their antibacterial activities since they were more potent than compound 15. Comparing with PTM, all the three compounds shared other similar interactions with the ecFabF(C163Q) residues. For example, they interacted with Thr270 through the ether oxygen, but lost the critical hydrogen bond with Ala309.

Docking studies of 6,7-dihydroplatensimycin 7 and compounds 9–12 were also performed according to the above procedures (Fig. 2 and S1†). Interestingly, the docking results

Fig. 1 Docking studies of PTM (a), 15 (b), 17 (c) and 25 (d) by MOE using ecFabF(C163Q) co-crystallized with PTM as template (PDB ID, 2GFX). The non-polar hydrogens of the ligands were hidden, in order to improve the clarity.

showed that 6,7-dihydroplatensimycin had a twisted chair conformation, slightly different from the chair conformation in its co-crystal structure with ecFabF(C163Q).¹⁵ In contrast, the conformation of compounds 9–12 deviated drastically from PTM and 6,7-dihydroplatensimycin, which lost the important interactions with Thr270, Ala309 and Arg206 as observed in the co-crystal structures of PTM or 6,7-dihydroplatensimycin with ecFabF(C163Q) (Fig. 2b and S1†).

Their energy minimal conformations were also calculated using the density function theory (Fig. 2c and S2†). The conformations of compounds 9–12 were quite different from PTM and 6,7-dihydroplatensimycin 7, and they could not be aligned together, especially in their ketolide part.

In order to compare their antibacterial activities, 6,7-dihydroplatensimycin and compounds 9–12 were prepared

according to the previous methods.¹⁴ 6,7-Dihydroplatensimycin was obtained efficiently by a Pd/C-catalyzed hydrogenation of PTM sodium salt. Treatment PTM with sodium borohydride yielded a mixture of compound 9 and three of its minor analogues 10–12. Interestingly compounds 9–12, when assayed as a mixture, were inactive against the tested *S. aureus* strains with a MIC > 64 $\mu\text{g mL}^{-1}$, while 6,7-dihydroplatensimycin was only slightly less active than PTM, similar to its reported MIC (Table 1).¹³ Therefore, the keto group might play an essential role to maintain the original active conformation with its target FabF, which was consistent with the previous report that the conformation of PTM ketolide has big effects on its activity.¹⁵

Experimental

General experimental procedure

The commercial reagents were used as received. All ^1H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz or 400 MHz spectrometer. Chemical shifts were reported in ppm relative to the internal standard tetramethylsilane ($\delta = 0$ ppm) for ^1H NMR and deuterio chloroform ($\delta = 77.00$ ppm) for ^{13}C NMR spectroscopy. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. HRMS spectra were recorded on a Bruker ULTRAFLEX III TOF/TOF 200 instrument. All compounds examined possessed a purity of at least 95%. Details are available in ESI†

6,7-Dihydroplatensimycin 7. Synthesis of 7 was based on the previous report.¹⁵ PTM (0.2 mmol, 88.0 mg) and NaOH (0.2 mmol, 8.0 mg) were dissolved in 1 mL water. To a solution of this mixture in 20 mL MeOH was added 10% Pd/C (15.0 mg) and stirred under H_2 atmosphere (1 atm) for 24 h. After Pd/C was removed by filtration, the filtrate was concentrated under reduced pressure to generate 7 in quantitative yields.

PTM derivatives 9–12. Synthesis of 9–12 was based on the previous report.¹⁴ To a solution of PTM (0.2 mmol, 88.0 mg) in 5 mL methanol was added sodium borohydride (5.0 mmol, 189.0 mg) in portion. The reaction mixture was stirred at room temperature for 1 h. After the reaction, 30 mL ethyl acetate, 30 mL H_2O and 1 mL acetic acid were added. The organic layer was washed with brine (3×15 mL) and H_2O (3×15 mL), and dried with sodium sulfate. The solvent was finally removed under reduced pressure to yield the mixture of PTM derivatives 9–12 in quantitative yields.

PTM derivative 13. To a solution of PTM (0.2 mmol, 88.0 mg) in methanol was added hydroxylamine hydrochloride (1.0 mmol, 70.0 mg). The reaction mixture was stirred at room temperature for 24 h. After the reaction, MeOH was removed by reduced pressure and the crude product was purified through silica flash chromatograph to yield PTM derivative 13 (81.0 mg, 83%).

PTM derivative 14. To a solution of 13 (0.2 mmol, 98.0 mg) in 30 mL MeOH was added NaOH (0.3 mmol, 12.0 mg). The reaction mixture was stirred at room temperature for 48

Fig. 2 Docking studies of 7 (a) and 9 (b) by MOE using ecFabF(C163Q) co-crystallized with PTM as template (PDB ID, 2GFX). Alignment of energy minimized conformations of PTM, compound 7, 9 and 10 generated from DFT calculation (c). The non-polar hydrogens of the ligands were hidden to improve the clarity.

h and isolated through preparative thin-layer chromatography (TLC) (developing solvents, ethyl acetate:acetic acid = 100:1) to yield derivative 14 (38.0 mg, 42%).

PTM derivative 15. To a solution of PTM (0.2 mmol, 88.0 mg) in alcohol (20 mL) was added methoxyammonium chloride (1.0 mmol, 83.0 mg) and triethylamine (1.0 mmol, 101.0 mg). The reaction mixture was stirred at reflux for 12 h. At the end of the reaction, the solvent was removed by reduced pressure and the crude product was purified through silica flash chromatograph to yield PTM derivative 15 (69.0 mg, 73%).

PTM derivative 16. The method of preparing derivative 16 is similar to that of 15, except that the reactant is *N*-ethylhydroxylamine hydrochloride (1.0 mmol, 97.0 mg). PTM derivative 16 was obtained (54.0 mg, 56%).

General procedure for the preparation of PTM derivatives 17–26. To a solution of PTM (0.2 mmol, 88.0 mg) and acyl hydrazine (2.0 mmol) in 20 mL alcohol was added catalytic amount of acetic acid. The reaction mixture was stirred at reflux for 24–96 h. At the end of the reaction, the solvent was removed under reduced pressure. The dried residues were dissolved in ethyl acetate, washed with 0.1 N hydrochloric acid and brine. The organic layer was dried with sodium sulfate and concentrated to generate the crude product. The crude product was separated with preparative TLC (developing solvents, ethyl acetate:acetic acid = 100:1) and then polyamide chromatography (elutes, water:ethanol = 9:1–7:1). Finally, the products were obtained by preparative high performance liquid chromatography.

The MIC determination²¹

In vitro bioassay for the tested compounds were carried out using *S. aureus*, *E. coli* and *K. pneumoniae*, following the standard procedure for agar dilution method.²¹ In brief, the bacteria were cultivated in LB medium and incubated for 16 h at 37 °C, 250 rpm, which were then diluted till the OD₆₀₀ reached to 0.25 ± 0.5, and then further diluted by 10⁴ fold. The diluted bacteria (2 µL, about 10⁴ colony forming units) were added to each LB plate containing different concentration of the test compounds (0.5–64 µg mL⁻¹), and the plates were incubated for 16 h at 37 °C. The MICs were determined when no growth of the tested bacteria was observed on the plates.

Stability assay of the PTM analogues in LB agar

PTM derivatives 15, 17, 21, 23 and 25 (0.1 mg mL⁻¹) were diluted in 5 mL of LB agar (1.5% v/v) to 8 µg mL⁻¹, and the LB agar plates were then incubated at 37 °C for 16 h. The LB agar containing the tested compounds were extracted by DCM (3 × 10 mL), dried with sodium sulfate and concentrated in vacuum. The resulting extracts were analyzed by ultra-performance liquid chromatography.

Molecular docking

The molecular modelling studies in this work were carried out on an Intel core i5 2.5 GHz processor, 4 GB memory with Windows 10 operating system using Molecular Operating En-

vironment (MOE 2010.06; Chemical Computing Group, Canada) as the computational platform. All the energy minimizations were performed with MOE until a RMSD gradient of 0.05 kcal mol⁻¹ Å⁻¹ with MMFF94x force-field and the partial charges were automatically calculated. The X-ray crystallographic structure of ecFabF(C163Q) complexed with PTM (PDB ID: 2GFX) was obtained from the Protein Data Bank. The enzyme was prepared for docking studies where: (i) errors in crystallographic structure were corrected with prepare structure step and water molecules were removed from the complex; (ii) hydrogen atoms and partial charges were added with Protonate 3D step; (iii) the target site was defined by selecting PTM as the template. Molecular docking simulations were performed using the MMFF94x force field and the “triangle matcher” method.

Density function calculation

All the density functional theory (DFT) calculations were carried out in the Gaussian 09 software package (Revision D.01).²³ The geometric structures of these compounds were fully optimized using the M06-2X functional method combined with the 6-31G(d) basis set. The frequency analysis was also performed based on the optimized structures to verify the energy minimum. All discussed energy values are the Gibbs free energies calculated at the temperature 298 K and the distances in angstrom.

Conclusions

In conclusion, 14 new ketolide modified PTM analogues were synthesized. Among these compounds, PTM derivative 17 showed the most potent activity against MRSA (MIC = 1.0–2.0 µg mL⁻¹), which was also stable under the assay conditions. In addition, most PTM derivatives have MIC values between 4.0–64.0 µg mL⁻¹ against various strains of *S. aureus*. The docking study revealed that the active PTM derivatives might have additional interactions with ecFabF(C163Q). The study of 6,7-dihydroplatensimycin 7 and the other PTM derivatives 9–12 further revealed that the keto group in the cyclohexenone was important to maintain the active conformation of PTM. Taken together, our results suggested that replacing the keto function group with certain oxime, hydrazine or hydrazide derivatives in the PTM cyclohexenone structure could still maintain the optimal terpene ketolide conformation against its target protein FabF, which thus provides a facile entry to diversify active PTM scaffolds through Schiff base formation.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported in parts by NSFC grants 81473124 (to Y. H.), the Chinese Ministry of Education 111 Project B0803420 (to Y. D.), NIH Grant GM114353 (to B. S.). We thank

the Center for Advanced Research in CSU for the HRMS and NMR experiments and Shenzhen Cloud Computing Center.

Notes and references

- 1 R. J. Heath and C. O. Rock, *Nat. Prod. Rep.*, 2002, **19**, 581–596.
- 2 J. Yao and C. O. Rock, *Biochimie*, 2017, **141**, 30–39.
- 3 H. T. Wright and K. A. Reynolds, *Curr. Opin. Microbiol.*, 2007, **10**, 447–453.
- 4 Z. Feng, D. Chakraborty, S. B. Dewell, B. V. B. Reddy and S. F. Brady, *J. Am. Chem. Soc.*, 2012, **134**, 2981–2987.
- 5 J. Wang, S. M. Soisson, K. Young, W. Shoop, S. Kodali, A. Galgoci, R. Painter, G. Parthasarathy, Y. S. Tang and R. Cummings, *Nature*, 2006, **441**, 358–361.
- 6 J. Wang, S. Kodali, S. H. Lee, A. Galgoci, R. Painter, K. Dorso, F. Racine, M. Motyl, L. Hernandez and E. Tinney, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 7612–7616.
- 7 E. Martens and A. L. Demain, *J. Antibiot.*, 2011, **64**, 705–710.
- 8 J. D. Rudolf, L. B. Dong and B. Shen, *Biochem. Pharmacol.*, 2017, **133**, 139–151.
- 9 K. C. Nicolaou, A. F. Stepan, T. Lister, A. Li, A. Montero, G. S. Tria, C. I. Turner, Y. Tang, J. Wang and R. M. Denton, *J. Am. Chem. Soc.*, 2008, **130**, 13110–13119.
- 10 K. Nicolaou, Y. Tang, J. Wang, A. F. Stepan, A. Li and A. Montero, *J. Am. Chem. Soc.*, 2007, **129**, 14850–14851.
- 11 K. C. Nicolaou, T. Lister, R. M. Denton, A. Montero and D. J. Edmonds, *Angew. Chem.*, 2007, **119**, 4796–4798.
- 12 K. P. Jang, C. H. Kim, S. W. Na, D. S. Jang, H. Kim, H. Kang and E. Lee, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2156–2158.
- 13 H. C. Shen, F. X. Ding, S. B. Singh, G. Parthasarathy, S. M. Soisson, S. N. Ha, X. Chen, S. Kodali, J. Wang and K. Dorso, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1623–1627.
- 14 S. B. Singh, K. B. Herath, J. Wang, N. Tsou and R. G. Ball, *Tetrahedron Lett.*, 2007, **48**, 5429–5433.
- 15 S. B. Singh, H. Jayasuriya, J. G. Ondeyka, K. B. Herath, C. Zhang, D. L. Zink, N. N. Tsou, R. G. Ball, A. Basilio and O. Genilloud, *J. Am. Chem. Soc.*, 2006, **128**, 11916–11920.
- 16 L. Qiu, K. Tian, J. Pan, L. Jiang, H. Yang, X. C. Zhu, B. Shen, Y. Duan and Y. Huang, *Tetrahedron*, 2017, **73**, 771–775.
- 17 L. Qiu, K. Tian, Z. Q. Wen, Y. C. Deng, D. D. Kang, H. Y. Liang, X. C. Zhu, B. Shen, Y. Duan and Y. Huang, *J. Nat. Prod.*, 2018, **81**, 316–322.
- 18 Y. Li, C. P. Zhao, H.-P. Ma, M.-Y. Zhao, Y.-R. Xue, X.-M. Wang and H. L. Zhu, *Bioorg. Med. Chem.*, 2013, **21**, 3120–3126.
- 19 Y. Zhou, Y. Luo, Y. S. Yang, L. Lu and H. L. Zhu, *MedChemComm*, 2016, **7**, 1980–1987.
- 20 H. Beraldo and D. Gambinob, *Mini-Rev. Med. Chem.*, 2004, **4**, 31–39.
- 21 I. Wiegand, K. Hilpert and R. E. Hancock, *Nat. Protoc.*, 2008, **3**, 163–175.
- 22 F. Ali, K. M. Khan, U. Salar, M. Taha, N. H. Ismail, A. Wadood, M. Riaz and S. Perveen, *Eur. J. Med. Chem.*, 2017, **138**, 255–272.
- 23 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09, Revision D.01*, Gaussian, Inc., Wallingford CT, 2013.