Bioorganic & Medicinal Chemistry Letters 21 (2011) 2297–2301

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and evaluation of thiophenyl derivatives as inhibitors of alkaline phosphatase

Lei Chang [a], Do Le Duy [b], Saida Mébarek [b], Florence Popowycz [a], Stéphane Pellet-Rostaing [a,c,*], Marc Lemaire [a,*], René Buchet [b,*]

[a] Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, UMR-CNRS 5246, Equipe Catalyse Synthèse Environnement, Université de Lyon, Université Claude Bernard–Lyon 1, Bâtiment Curien, 43 Bd du 11 Novembre 1918, F-69622 Villeurbanne, France
[b] Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, UMR-CNRS 5246, Equipe Organisation et Dynamique des Membranes Biologiques, Université de Lyon, Université Claude Bernard–Lyon 1, Bâtiment Raulin, 43 Bd du 11 Novembre 1918, F-69622 Villeurbanne, France
[c] Institut de Chimie Séparative de Marcoule, UMR-CNRS 5257, Site de Marcoule, Université Montpellier 2, F-30207 Bagnols sur Cèze, France

ARTICLE INFO

Article history:
Received 9 February 2011
Revised 21 February 2011
Accepted 23 February 2011
Available online 21 March 2011

Keywords:
Thiophene
Alkaline phosphatase
Inhibitor
Calcification disease

ABSTRACT

Pathological calcifications induced by deposition of basic phosphate crystals or hydroxyapatite (HA) on soft tissues are a large family of diseases comprising of ankylosing spondylitis (AS), end-stage osteoarthritis (OA) and vascular calcification. High activity of tissue non-specific alkaline phosphatase (TNAP) is a hallmark of pathological calcifications induced by HA deposition. The use of TNAP inhibitor is a possible therapeutic option to address calcific diseases produced by HA deposition on soft tissues. We report the synthesis of a series of thiopheno-imidazo[2,1-*b*]thiazole derivatives which were evaluated as potential inhibitors of TNAP displaying a large range of IC_{50} at pH 10.4 (from 42 ± 13 μM to more than 800 μM).

Calcific diseases are a large family of diseases, affecting not only skeletal (bones and joints) but also non-skeletal tissues. Among calcific diseases, there are pathological calcifications inducing hydroxyapatite deposition in soft tissues such as end-stage osteoarthritis (OA) occurring in joint cartilage, medial artery calcification occurring in tunica media, ankylosing spondylitis (AS) occurring in tendons or ligaments or tumor calcification (as in breast cancer).[1] Osteoarthritis (OA) is the most common form of arthritis in adults.[2] OA is characterized by cartilage loss, new bone formation at the margin of the joints (osteophytes), change of subchondral bone and recurrent synovitis. Advanced OA is associated with calcification involving hyaline articular cartilage matrix.[3] Basic calcium phosphates particularly hydroxyapatite (HA) are predominant crystals in OA cartilage[2,4,5] and were found in all end-stage OA patients.[6] Vascular calcification is associated with increased risk for cardiovascular disease and mortality. Some of the risk factors are the classic ones associated with coronary artery disease in the general population (e.g., hypertension, diabetes, dyslipidemia), whereas others are specific to patients with end-stage renal disease (chronic kidney disease or CKD).[7] As most age-related diseases, calcific diseases are multi-factorial and are relatively complex. Since most of the calcific diseases induce- or promote-inflammatory reaction, the use of anti-inflammatory drugs has been a successful strategy to relieve pain for patients affected by calcific diseases. The healthcare management has clearly improved since the administration of anti-TNF drugs like infliximab,[8,9] etanercept[10] and adalimumab[11] affording AS patients a better quality of life. Reduction of mortality in CKD patients can be achieved with anti-inflammatory strategies.[12] Anti-inflammatory drugs such as analgesics or non-steroidal anti-inflammatory drugs and lifestyle modifications can relieve pain and symptoms for patients having OA.[13] Although most of anti-inflammatory drugs are effective to relieve pain, they do not cure the pathological calcifications and they do not afford a complete remission for all patients. Recently, more attention has been devoted to the development of drug therapies directly dealing with the calcification process.[14–17] The cells in soft tissues (tendon or ligament cells for AS; chondrocytes for end-stage OA; vascular smooth muscle cells for vascular calcification) which do not mineralize under physiological conditions become mineral competent under pathological conditions. Increased expression of tissue non-specific alkaline phosphatase (TNAP) in mineral competent cells accelerates calcification. The main function of TNAP is to hydrolyze extracellular pyrophosphate (ePP_i), an inhibitor of HA formation and

* Corresponding authors. Tel.: +3 346 633 9308; fax: +3 346 679 7611 (S.P.-R.); tel.: +3 347 243 1409; fax: +3 347 243 1408 (M.L.); tel.: +3 347 243 1542; fax: +3 347 243 1543 (R.B.).
E-mail addresses: stephane.pellet-rostaing@cea.fr (S. Pellet-Rostaing), marc.lemaire@univ-lyon1.fr (M. Lemaire), rbuchet@univ-lyon1.fr (R. Buchet).

0960-894X/$ - see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2011.02.089

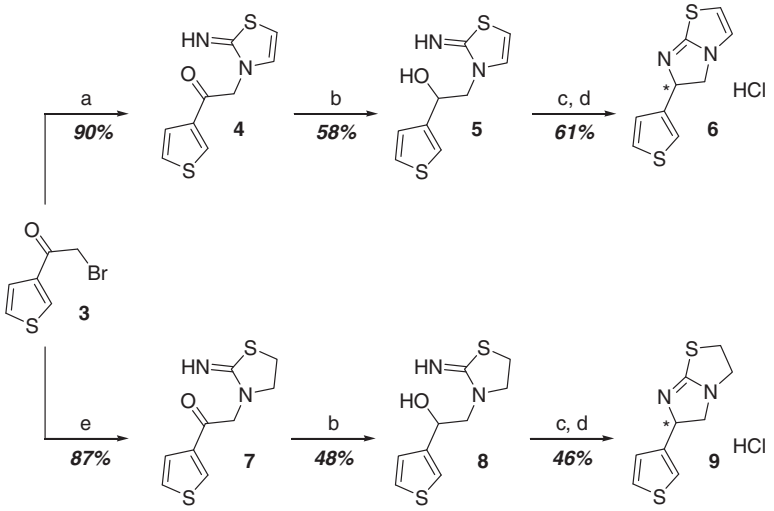
Figure 1. Structure of known TNAP inhibitors.

calcification, suggesting that ePP_i deficiency is a potential therapeutic target.[15] Inhibitors of TNAP could increase the local amount of ePP_i and slowing down the calcification. Recently, we reported the synthesis and evaluation of benzo[*b*]thiophene derivatives as inhibitors of TNAP and of intestinal alkaline phosphatases.[14] The most promising inhibitors were those which inhibited specifically intestinal alkaline phosphatase but two racemic TNAP inhibitors were found having similar inhibition property (benzo[*b*]thiopheno-2,3-dehydrotetramisole **2a**) or better inhibition property (benzo[*b*]thiopheno-tetramisole **2b**) than the enantiomeric levamisole **1** (Fig. 1).[14]

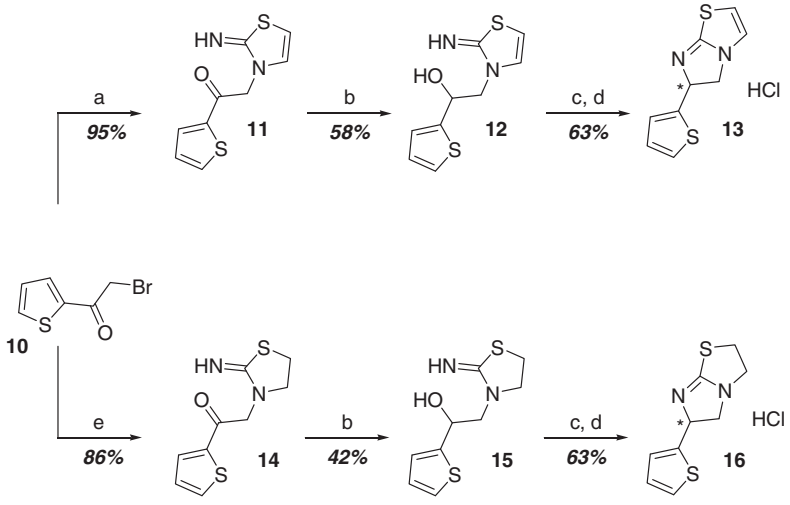
In this Letter, we report the synthesis of several thiophene inhibitors of TNAP. Their IC_{50} are compared with that of levamisole, a specific inhibitor of TNAP which has been used for the treatment of rheumatoid arthritis,[18–20] an autoimmune disease, unrelated to OA, which eventually leads to synovial inflammation and destruction of joint architecture. Therapeutical use of levamisole is presently limited due to skin rashes and agranulocytosis.[21,22]

Replacement of benzo[*b*]thiophene group could lead to stronger inhibitory activity as suggested by our findings on benzo[*b*]thiopheno-tetramisole derivatives. Following this strategy, four new thiophenyl derivatives (**6**, **9**, **13** and **16**) substituted either in C-2 or in C-3 positions were prepared, bearing the imidazo[2,1-*b*]thiazole scaffold. The synthetic chemistry used for the preparation of these compounds is described in Schemes 1 and 2. Final compounds **6** and **9** were obtained in four steps from the known 3-(2-bromoacetyl)thiophene **3**.

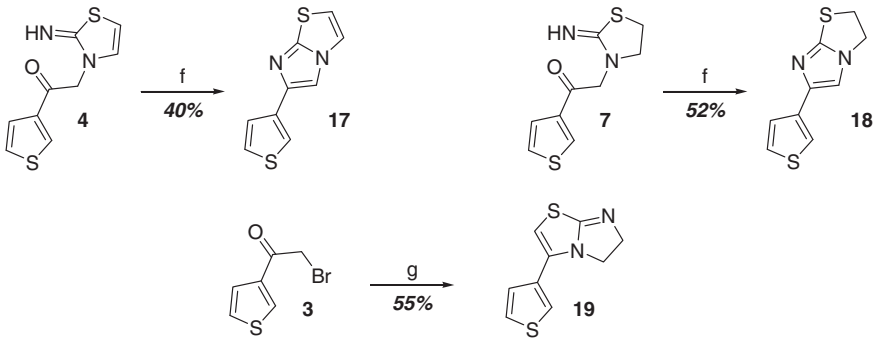
Compound **3** was previously synthesized from commercially available thiophene following the conditions already reported in



Scheme 1. Synthetic pathway to 3-substituted thiophenes. Reagents and conditions: (a) 2-aminothiazole (1.1 equiv), 2-propanol, reflux, 1 h; (b) $NaBH_4$ (2.2 equiv), MeOH, rt, 14 h; (c) (i) $SOCl_2$, reflux, 30 min, (ii) 10% Na_2CO_3 aq-DCM, reflux, 2 h; (d) HCl (2 M ether), DCM; (e) 2-aminothiazoline, 2-propanol, reflux, 1 h.



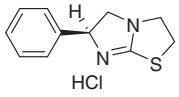
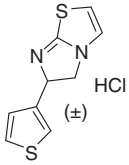
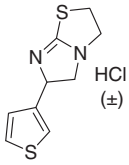
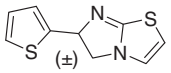
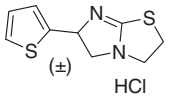
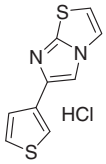
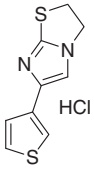
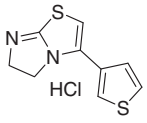
Scheme 2. Synthetic pathway to 2-substituted thiophenes. Reagents and conditions: (a) 2-aminothiazole (1.1 equiv), 2-propanol, reflux, 1 h; (b) $NaBH_4$ (2.2 equiv), MeOH, rt, 14 h; (c) (i) $SOCl_2$, reflux, 30 min, (ii) Na_2CO_3 aq-DCM, reflux, 2 h; (d) HCl (2 M ether), DCM; (e) 2-aminothiazoline, 2-propanol, reflux, 1 h.



Scheme 3. Other 3-substituted thiophenes. Reagents and conditions: (f) 2 N aq HBr, reflux, 2 h; (g) 2-imidazolidinethione (1.1 equiv), MeCN, reflux, 2 h then anhydrous ethylene glycol dimethyl ether, reflux, 2 h.

the litterature.[23] Nucleophilic substitution on molecule **3** was carried out under reflux in the presence of 2-aminothiazole, in isopropanol during one hour leading to iminothiazoline **4** in 90% yield (Scheme 1). Subsequent reduction of ketone by sodium borohydride in anhydrous methanol at room temperature provided intermediate alcohol **5**. Treatment of compound **5** in the presence of thionyl chloride at reflux allowed the cyclized product which upon treatment with HCl (2 M in ether) afforded the corresponding hydrochloride salt **6**. Compound **6** was prepared in a four steps sequence from **3** in an overall yield of 32%. Following the similar

Table 1
IC_{50} values (three independent measurements) determined at 37 °C at pH 10.4 (optimal conditions for the biological assay) and at pH 7.8 (physiological conditions)

General structures	Compound number	IC_{50} (μM) ± SD pH 10.4	IC_{50} (μM) ± SD pH 7.8
	**1** Levamisole	93 ± 23	78 ± 17
	**6**	42 ± 13	84 ± 9
	**9**	408 ± 168	192 ± 40
	**13**	331 ± 168	260 ± 36
	**16**	>800	>800
	**17**	>800	>800
	**18**	>800	>800
	**19**	>800	>800

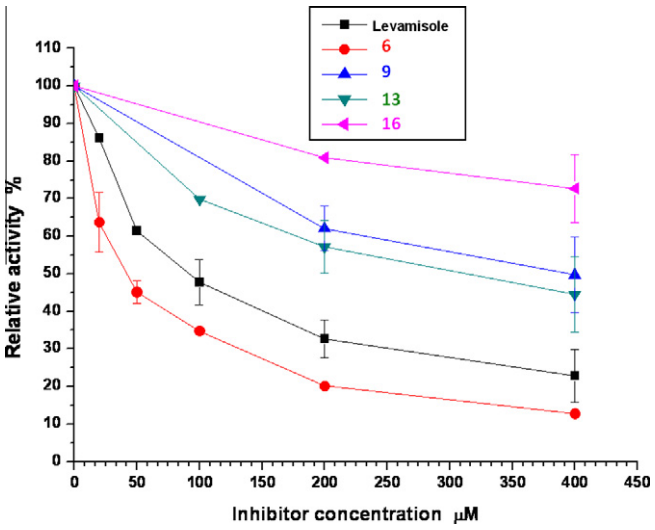


Figure 2. Relative activities of TNAP determined at 37 °C and pH 10.4 as function of concentration of levamisole and selected thiopheno-imidazo[2,1-*b*]thiazole derivatives.

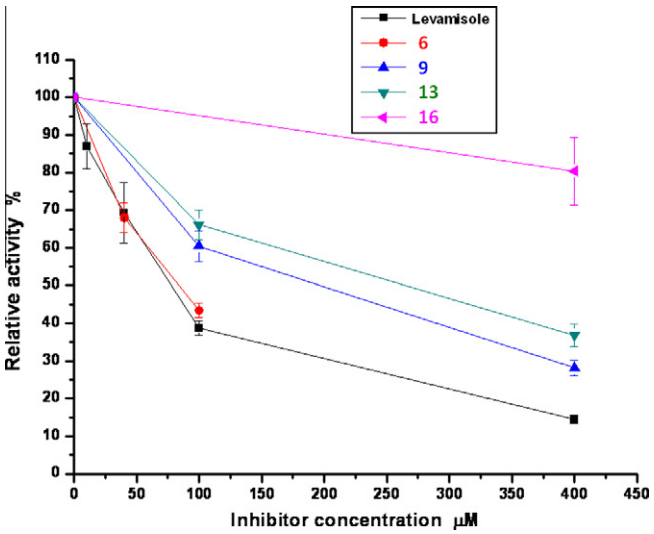


Figure 3. Relative activities of TNAP determined at 37 °C and pH 7.8 as function of concentration of levamisole and selected thiopheno-imidazo[2,1-*b*]thiazole derivatives.

strategy, the condensation of **3** with the 2-aminothiazoline gave the intermediate **7** which was converted into the tetramisole derivative **9** with a global yield of 19% (Scheme 1).

The 2-substituted thiophene analogues were prepared in a similar synthetic pathway, 2,3-dehydrotetramisole and tetramisole analogues **13** and **16** respectively prepared in global yield of 35% and 23% (Scheme 2).

The imidazo[2,1-*b*]thiazole **17** and its corresponding 2,3-dehydro- derivative **18** were prepared from 3-(2-bromoacetyl)-thiophene with 2-aminothiazole and 2-aminothiazoline, respectively, through intermediates **4** and **7** (Scheme 3). Treatment in acidic medium allowed the intramolecular condensation and aromatization of the ring formed. Other derivative of 2,3-dehydrotetramisole **19** was obtained from 3-(2-bromoacetyl)thiophene **3** upon treatment with 2-imidazolidinethione.

The inhibition of TNAP activity has been tested for a series of thiophenyl tetramisole derivatives at pH 10.4. Best compound **6** as a racemic mixture, inhibited TNAP at lower concentration (IC_{50} = 42 ± 13 μM) than the *S* enantiomer of levamisole **1** (IC_{50} = 93 ± 23 μM) (Table 1). Other derivatives such as **9** (IC_{50} = 408 ± 168 μM) and **13** (IC_{50} = 331 ± 168 μM) inhibited moderately TNAP as compared with levamisole. Other compounds such as **16–19** did not inhibit significantly (IC_{50} >800 μM) (Table 1, Fig. 2). Values determined at pH 7.8 (Fig. 3) are less indicative since the assay is based on the detection of nitrophenolate (pK_a = 7) but pointed out that inhibitors kept their properties at neutral pH. At pH 7.8, the apparent IC_{50} of racemic compound **6** (IC_{50} = 84 ± 9 μM) is similar to that of levamisole **1** (IC_{50} = 78 ± 17 μM) (Table 1). The thiophene inhibitors obtained here are less potent than pyrazole derivatives having lower IC_{50}.[16,17] IC_{50} determined by our team are overestimated as compared with those reported[16,17]due to distinct origin of TNAP and biological assay. K_i of levamisole also amounted to 93 ± 4 μM,[14] while it was 16 μM for the reported value.[16] TNAP (final TNAP concentration was 6.93–11.4 μg mL^{-1}) was from porcine origin in our UV–vis assays, while TNAP was from recombinant origin in the luminescent assays.[16,17]

There is an urgent need to obtain other TNAP inhibitors since there are very few.[16,24] One of the problem encountered with inhibitors is their specificity. For example, levamisole not only inhibited TNAP but affected indirectly Ca^{2+} or Pi transport.[24] Therefore, the synthesis of other TNAP inhibitors is a necessity not only to design a possible drug therapy to cure pathological calcification but also to characterize distinct aspects of mineralization mechanisms.

A large range of IC_{50} (from 42 ± 13 μM to more than 800 μM) is obtained with a single chemical modification on the same chemical motif, thiopheno-imidazo[2,1-*b*]thiazole. The racemic thiophenyl analogue of levamisole (**6**·HCl) with apparent IC_{50} = 42 ± 13 μM (*n* = 3, pH 10.4) is twice more potent than enantiomeric levamisole **1** (IC_{50} = 93 ± 23 μM) (as determined with porcine kidney TNAP), indicating some potential to synthesize and optimize enantiomeric thiophenyl derivatives for therapeutic applications to treat pathological calcifications. Most of the inhibitors were not very soluble in water medium. Enlarging chemical and physical properties of inhibitors to obtain the best pharmacokinetic parameters as well as to increase the selectivity into extra cellular medium, synthesis of water-soluble inhibitors are a necessary step toward generation of a series of inhibitors.

Acknowledgment

The authors deeply acknowledge Professor David Magne (ICBMS-Université de Lyon) for fruitful discussion.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.089.

References and notes

1. Anderson, H. C. *Curr. Opin. Orthop.* **2007**, *18*, 428.
2. Kalya, S.; Rosenthal, A. K. *Curr. Opin. Rheumatol.* **2005**, *17*, 325.
3. Steinbach, L. S.; Resnick, D. *Curr. Probl. Diagn. Radiol.* **2000**, *29*, 209.
4. McCarthy, G. M.; Cheung, H. S. *Curr. Rheumatol. Rep.* **2009**, *11*, 141.
5. Ea, H. K.; Lioté, F. *Curr. Opin. Rheumatol.* **2009**, *21*, 150.
6. Fuerst, M.; Bertrand, J.; Lammers, L.; Dreier, R.; Echtermeyer, F.; Nitschke, Y.; Rutsch, F.; Schäfer, F. K. W.; Niggemeyer, O.; Steinhagen, J.; Lohmann, C. H.; Pap, T.; Rüther, W. *Arthritis Rheum.* **2009**, *60*, 2694.
7. Ehrlich, J. E.; Rumberger, J. A. *Dialysis Transplant.* **2004**, *33*, 306.
8. Van der Heijde, D.; Dijkmans, B.; Geusens, P.; Sieper, J.; DeWoody, K.; Williamson, P.; Braun, J. *Arthritis Rheum.* **2005**, *52*, 582.
9. Antoni, C.; Krueger, G. G.; de Vlam, K.; Birbara, C.; Beutler, A.; Guzzo, C.; Zhou, B.; Dooley, L. T.; Kavanaugh, A. *Ann. Rheum. Dis.* **2005**, *64*, 1150.

10. Brandt, J.; Khariouzov, A.; Listing, J.; Haibel, H.; Sörensen, H.; Grassnickel, L.; Rudwaleit, M.; Sieper, J.; Braun, J. *Arthritis Rheum.* **2003**, *48*, 1667.
11. Van der Heijde, D.; Kivitz, A.; Schiff, M. H.; Sieper, J.; Dijkmans, B. A.; Braun, J.; Dougados, M.; Reveille, J. D.; Wong, R. L.; Kupper, H.; Davis, J. C., Jr. *Arthritis Rheum.* **2006**, *54*, 2136.
12. Stinghen, A. M. S.; Bucharles, S.; Riella, M. C.; Pecoits-Filho, R. *Blood Purif.* **2010**, *29*, 114.
13. Crosby, J. *J. Fam. Pract.* **2009**, *58*, 354.
14. Li, L.; Chang, L.; Pellet-Rostaing, S.; Liger, F.; Lemaire, M.; Buchet, R.; Wu, Y. *Bioorg. Med. Chem.* **2009**, *17*, 7290.
15. Narisawa, S.; Harmey, D.; Yadav, M. C.; O'Neill, W. C.; Hoylaerts, M. F.; Millán, J. L. *J. Bone Miner. Res.* **2007**, *22*, 1700.
16. Lanier, M.; Sergienko, E.; Simão, A. M.; Su, Y.; Chung, T.; Millán, J. L.; Cashman, J. R. *Bioorg. Med. Chem.* **2010**, *18*, 573.
17. Dahl, R.; Sergienko, E. A.; Su, Y.; Mostofi, Y. S.; Yang, L.; Simão, A. M.; Narisawa, S.; Brown, B.; Mangravita-Novo, A.; Vicchiarelli, M.; Smith, L. H.; O'Neill, W. C.; Millán, J. L.; Cosford, N. D. *J. Med. Chem.* **2009**, *52*, 6919.
18. Schuermans, Y. *Lancet* **1975**, *1*, 111.
19. McGill, P. E. *Lancet* **1976**, *2*, 149.
20. Miller, B.; De Merieux, P.; Srinivasan, R.; Clements, P.; Fan, P.; Levy, J.; Paulus, H. E. *Arthritis Rheum.* **1980**, *23*, 172.
21. Rosenthal, M.; Breysse, Y.; Dixon, A. S.; Franchimont, P.; Huskisson, E. C.; Schmidt, K. L.; Schuermans, Y.; Veys, E.; Vischer, T. L.; Janssen, P. A.; Brugmans, J.; de Crepe, J.; Symoens, J.; Macnair, A. L.; Amery, W. K.; Veys, E. *Lancet* **1977**, *1*, 904.
22. Mielants, H.; Veys, E. M. *J. Rheumatol.* **1978**, *5*, 77.
23. Macdowell, D. W. H.; Greenwood, T. D. *J. Heterocycl. Chem.* **1965**, *2*, 44.
24. Li, L.; Buchet, R.; Wu, Y. *J. Inorg. Biochem.* **2010**, *104*, 446.