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Synthesis and biological activity of *N*-aryl-2-aminothiazoles: potent pan inhibitors of cyclin-dependent kinases

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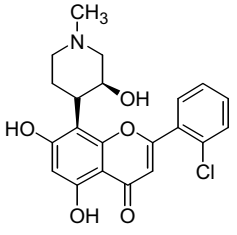
Abstract—*N*-Aryl aminothiazoles **6**–**9** were prepared from 2-bromothiazole **5** and found to be CDK inhibitors. In cells they act as potent cytotoxic agents. Selectivity for CDK1, CDK2, and CDK4 was dependent of the nature of the *N*-aryl group and distinct from the CDK2 selective *N*-acyl analogues. The *N*-2-pyridyl analogues **7** and **19** showed pan CDK inhibitory activity. Elaborated analogues **19** and **23** exhibited anticancer activity in mice against P388 murine leukemia. The solid-state structure of **7** bound to CDK2 shows a similar binding mode to the *N*-acyl analogues.

Cyclin-dependent kinases (CDKs) are a prominent family of protein kinases, which play a key role in the growth, development, proliferation, and death of eukaryotic cells.[1] Along with their regulatory subunit cyclins, CDKs serve the function of orderly coordinating events which move cells through the cell cycle and insure the genetic integrity of daughter cells. Due to their identified role as drivers of aberrant cell growth and division in a number of cancers, drug discovery programs have directed a major effort toward the identification of small molecule inhibitors of CDKs as potential antitumor therapeutic agents.[2,3]

Flavopiridol, **1**, is a pioneering benchmark CDK inhibitor, which shows inhibitory activity against a broad range of kinases including CDK1, CDK2, and CDK4 and is currently in Phase 2 clinical trials as an antitumor agent.[4] We recently reported on novel *N*-acyl-2-aminothiazoles **2** and **3**, which were potent CDK inhibitors.[5] In contrast to flavopiridol, **2** and **3** exhibited selective inhibition of CDK2. Shown in Table 1 is the inhibitory activity of **1**–**3** in cell-free enzyme assays and their cytotoxic activity against a human ovarian (A2780) cancer cell line.



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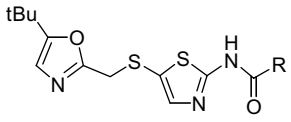

2: R=CH$_3$
3: R=CH$_2$-3-pyridyl

Table 1. Structure and biological activity of CDK inhibitors[a]

Compds	CDK1/cycB IC_{50}, nM	CDK2/cycE IC_{50}, nM	CDK4/cycD IC_{50}, nM	A2780 Cytotox IC_{50}, nM
1	30	170	100	71
2	40	5	690	50
3	80	5	1090	240

[a] See Ref. 5b for description of biological assays.

Herein we report on the synthesis, in vitro CDK inhibitory activity and in vivo anticancer activity against P388 murine leukemia of a series of *N*-aryl aminothiazoles, which show a CDK selectivity profile distinct from *N*-acyl analogues such as **2** and **3**.

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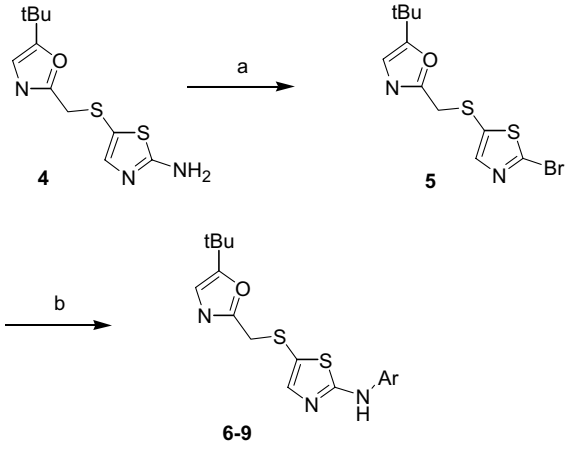


Scheme 1. Synthesis of *N*-aryl analogues **6**–**9**. Reagents and conditions: (a) $CuBr_2$ (1.2 equiv), *t*-BuONO (1.5 equiv), MeCN, 0–25 °C, 43%; (b) NaH (3–5 equiv), $ArNH_2$ (3–5 equiv)/THF, 55 °C then 0–25 °C, see Table 2 for yields.

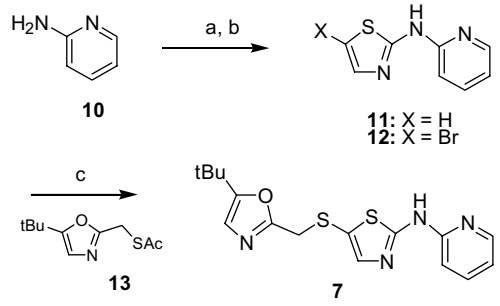
Table 2. Structure and synthesis of *N*-aryl aminothiazoles

Compd	Ar	Yield, %
6	Phenyl	17
7	2-Pyridyl	20
8	3-Pyridyl	14
9	4-Pyrimidinyl	40

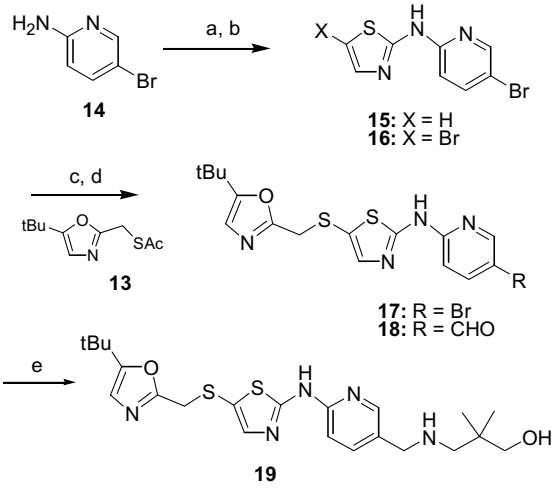
The *N*-aryl analogues **6**–**9** were prepared from the previously reported amine **4** via intermediate bromide **5** as shown in Scheme 1.[5,6] As indicated, treatment of **4** with *tert*-butyl nitrite in the presence of copper(II) bromide afforded the 2-bromo derivative **5** in moderate yield.[7] Bromide **5** was coupled with simple aryl amines by initially generating excess amine anion with sodium hydride in THF at 55 °C and then addition of **5** at 0–25 °C. The yields as indicated in Table 2 were low to moderate. The major side products appeared to result from competitive deprotonation of the acidic protons on the methylene group adjacent to the sulfur.

Alternatively, as shown in Scheme 2, the 2-pyridyl analogue **7** was prepared in improved yield by initially coupling excess 2-aminopyridine anion with 2-bromothiazole to afford **11**. Thiazole **11** was then brominated selectively at the 5-position to give **12**. Substitution at the 5-position with the in situ generated thiolate from acetate **13**[8] afforded **7** in 34% overall yield. The 2-pyridyl inhibitor **19** was available in a similar sequence from commercial 2-amino-5-bromopyridine, **14**, as the starting amine. As shown in Scheme 3, **14** was converted to bromide **17**. Deprotonation followed by metal–halogen exchange and quenching the resulting anion with DMF afforded aldehyde **18** in moderate yield. Aldehyde **18** was then treated with excess 3-amino-2,2-dimethylpropanol under standard reductive amination conditions to give **19**.[9]

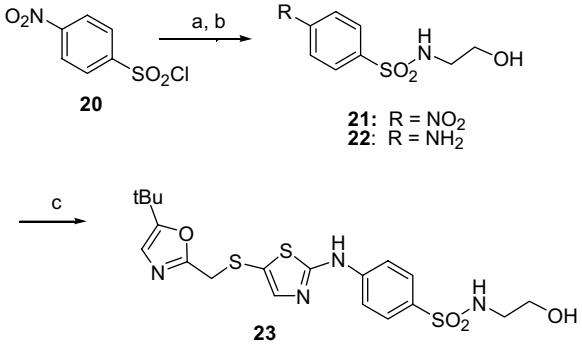
Finally, the 4-sulfonamide substituted *N*-phenyl analogue **23** was prepared in 57% yield, as shown in



Scheme 2. Synthesis of *N*-aryl analogue **7**. Reagents and conditions: (a) NaH (1 equiv), THF, 55 °C then 2-bromothiazole (0.33 equiv), 0–55 °C, 53%; (b) Br_2 (1.2 equiv), HOAc, 25 °C, 95%; (c) NaOH, $NaHCO_3$, aq MeOH, 25–60 °C, 68%.



Scheme 3. Synthesis of *N*-aryl analogue **19**. Reagents and conditions: (a) NaH (1 equiv), THF, 55 °C then 2-bromothiazole (0.33 equiv), 25–65 °C, 33%; (b) Br_2 (1.2 equiv), HOAc, 25 °C, 98%; (c) NaOH, aq MeOH, 25 °C then DMF, 60 °C, 57%; (d) *t*-BuMgCl (1.2 equiv) then *t*-BuLi (2.9 equiv), THF, −78 °C followed by DMF, 44%; (e) 3-amino-2,2-dimethylpropanol (8 equiv), $NaBH(OAc)_3$ (13 equiv), HOAc, THF, 70%.



Scheme 4. Synthesis of substituted *N*-phenyl aminothiazole **23**. Reagents and conditions: (a) 2-aminoethanol (1.3 equiv), Et_3N, 0–25 °C, 70%; (b) 10% Pd/C, H_2 (1 atm), HOAc/THF (1:1), 84%; (c) bromothiazole **5** (0.2 equiv), DMA, 145 °C, 5.5 h, 57%.

Scheme 4, by heating an excess of free amine **22** with bromide **5** in dimethylacetamide (DMA). The reaction conditions are a modification of the initial low yield

conditions in which the amine anion was generated prior to addition of the bromide **5**. Amine **22** was available in two-steps from phenylsulfonyl chloride **20** by coupling with 2-aminoethanol followed by reduction of the nitro group under palladium catalyzed hydrogenation conditions.

The three-dimensional solid-state structure of **7** in complex with CDK2 was determined by X-ray crystallography. Crystals were obtained by incubating the inhibitor (72 h) with crystalline protein in the absence of cyclin. The crystal structure, shown in the upper panel of Figure 1, confirmed that **7** binds in the ATP-binding site in the same folded conformation seen previously with the *N*-acyl aminothiazole chemotype (see overlay in the lower panel of Fig. 1).[5,10] In this conformation the *tert*-butyl oxazole ring folds back toward the thiazole core and into the ribose pocket rather than extending toward Phe-80. Clear hydrogen bonding interactions are not seen between the oxazole ring and the protein. Important hydrogen bonds between the amide backbone atoms of Leu-83 and both the thiazole nitrogen and exocyclic amine proton are clearly discernable. Finally and importantly, the *N*-aryl ring extends toward the exterior of the protein, which should allow for variable substitution *para* to the amino group. The origins for the differences in CDK enzyme inhibitory selectivity between the *N*-acyl and *N*-aryl series appear to be subtle and are not conclusively apparent from the structural data. However, they clearly are not the result of a change in hydrogen bonding interactions or a major change in binding mode.

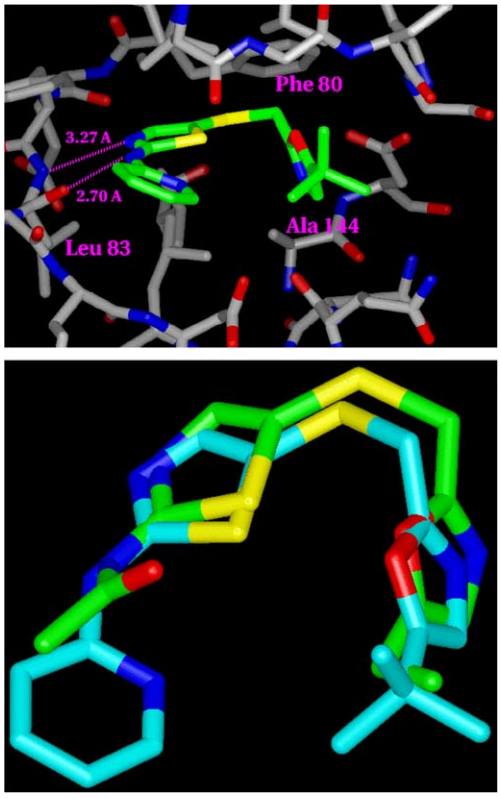


Figure 1. Upper panel: Solid-state structure of *N*-aryl aminothiazole **7** bound in the ATP-pocket of CDK2 (no cyclin). The inhibitor carbon atoms are shown in green, the nitrogen atoms are shown in blue, oxygen atoms are shown in red, and the sulfur atoms are shown in yellow. The protein carbons are in gray. Hydrogen bonds are shown by the magenta dotted lines. Lower panel: Overlay of *N*-aryl aminothiazole **7** with *N*-acyl aminothiazole **2** (*t*-butyl group replaced by an ethyl group). Inhibitor **7** is depicted with aqua colored carbon atoms and the ethyl analogue of **2** is depicted with green carbon atoms.

N-Aryl aminothiazoles were initially evaluated in a cell-free enzyme assay for inhibition of CDK1/cyclin B, CDK2/cyclin E, and CDK4/cyclin D induced phosphorylation of RB protein and subsequently for cytotoxicity in whole cells. An ovarian cancer cell line, A2780, was utilized in the cellular cytotoxicity assay. The results of these assays are shown in Table 3. Compounds **6**–**9** were all potent inhibitors of CDKs in the enzyme assay with a selectivity profile distinct from the *N*-acyl analogues (e.g., **2** and **3**). Whereas the *N*-acyl analogues exhibited selectivity for CDK2 over both CDK1 and CDK4, the *N*-aryl analogues generally showed diminished selectivity for CDK2 over CDK1 and CDK4. In the case of 2-pyridyl analogue **7**, potent inhibition of CDK1, CDK2, and CDK4 was observed.

Aminothiazoles **6**–**9** also exhibited potent cytotoxic activity in A2780 cells. Compound **9** was evaluated at its maximum tolerated dose (MTD) of 45 mg/kg for in vivo anticancer efficacy in mice against P388 murine leukemia. In the P388 model, drug was dosed intraperitoneally (ip) in immunocompetent mice immediately after tumor cells were implanted (ip), then once a day for 7 days (qdx7). The anticancer efficacy was measured as an increase in lifespan and the data are expressed as a ratio of lifespan of drug treated group (T) versus control group (C). The results indicated that despite its highly potent in vitro CDK inhibitory and cytotoxic activity, **9** was not active in vivo (%T/C = 110 for inhibitor **9**, active is defined as %T/C > 125) when increased lifespan was utilized as a criteria. Based on work with the *N*-acyl analogues we speculated that these compounds may be highly protein bound resulting in low levels of circulating free drug. In addition, poor solubility may be a

Table 3. CDK inhibitory activity of *N*-aryl aminothiazoles **6**–**9**[a]

Compd	Ar	CDK1/cycB IC_{50}, nM	CDK2/cycE IC_{50}, nM	CDK4/cycD IC_{50}, nM	A2780 Cytotox IC_{50}, nM
6	Phenyl	12	4	78	140
7	2-Pyridyl	4	2	9	10
8	3-Pyridyl	10	3	130	50
9	4-Pyrimidinyl	7	1	30	3

[a] See Ref. 5b for description of biological assays.

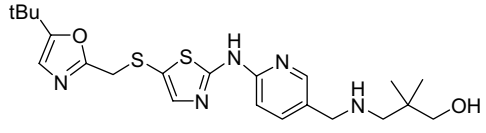
Table 4. CDK inhibitory and anticancer activity of *N*-aryl aminothiazoles[a]

Compd	CDK1/cycBIC$_{50}$, nM	CDK2/cycEIC$_{50}$, nM	CDK4/cycDIC$_{50}$, nM	A2780 CytotoxIC$_{50}$, nM	% Protein binding[b]	P388 %T/C
19	18	3	26	3	90	156 @ 4 mg/kg
23	10	2	233	69	97	180 @ 90 mg/kg

[a] See Ref. 5b for description of biological assays.
[b] Determined by equilibrium dialysis in mouse serum.

significant factor in the lack of anticancer activity for this agent. Following a strategy employed for the *N*-acyl aminothiazoles we sought to introduce polar functional groups into the molecule as a method of both increasing solubility and exposure of free drug.[5] Modeling and crystallographic studies (see above) had indicated that substituents *para* to the amino group on the aryl ring would be directed toward the exterior of the protein and thus should be compatible with maintaining CDK inhibitory activity. Based, in part, on modeling experiments compounds **19** and **23** with basic amine and alcohol side chains, respectively, were prepared and found to mirror the in vitro CDK inhibitory activity of their unsubstituted counterparts **6** and **7** (see Table 4). Analysis indicated serum protein binding at 90% and 97% for **19** and **23**, respectively. Examination in the P388 model showed that in contrast to **9**, both **19** and **23** were effective in significantly increasing lifespan. Administered at their MTD 2-pyridyl analogue **19** increased lifespan by 56% while 2-phenyl analogue **23** increased lifespan by 80%.

In summary, *N*-aryl aminothiazoles **6**–**9** were prepared and found to be inhibitors in vitro of CDK1, CDK2, and CDK4. In cells they acted as potent cytotoxic agents. Selectivity for CDK1, CDK2, and CDK4 was dependent on the nature of the *N*-aryl group and was distinct from the CDK2 selective *N*-acyl analogues. The *N*-2-pyridyl analogues **7** and **19** exhibited a pan-like CDK inhibitory profile. Analogues **19** and **23** with polar substituents on the aryl ring afforded inhibitors that were also efficacious in vivo as anticancer agents. For example, **19** (BMS-357075) was a potent inhibitor of CDK1, CDK2, and CDK4 and produced a 56% increase in survival time versus untreated control against P388 murine leukemia in mice (Fig. 2).



19 (BMS-357075)
CDK1/cycB IC_{50} = 18 nM
CDK2/cycE IC_{50} = 3 nM
CDK4/cycD IC_{50} = 26 nM
A2780 cytotox IC_{50} = 3 nM
P388 %T/C= 156

Figure 2. Summary of biological data for aminothiazole pan-CDK inhibitor **19**.

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References and notes

1. (a) Hunter, T.; Pines, J. *Cell* **1994**, *79*, 573; (b) Sherr, C. *Science* **1996**, *274*, 1672; Review: (c) Pines, J. *Seminars Cancer Biol.* **1994**, *5*, 305.
2. (a) Webster, K. R. *Exp. Opin. Invest. Drugs* **1998**, *7*, 1; (b) Webster, K. R.; Kimball, S. D. *Emerging Drugs* **2000**, *5*, 45; (c) Kimball, S. D.; Webster, K. R. *Annu. Rpt. Med. Chem.* **2001**, *36*, 139; (d) Sausville, E. A. *Trends Mol. Med.* **2002**, *8*(4, Suppl.), S32; (e) Huwe, A.; Mazitschek, R.; Giannis, A. *Angew. Chem., Int. Ed.* **2003**, *42*, 2122.
3. (a) Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J.; Donella-Deana, A.; Pinna, L.; Letham, D. S.; Kato, J.; Detivaud, L.; Leclerc, S.; Meijer, L. *Eur. J. Biochem.* **1994**, *224*, 771; (b) Abraham, R.; Acquarone, M.; Andersen, A.; Asensi, A.; Belle, R.; Berger, F.; Bergounioux, C.; Brun, G.; Buquet-Fagot, C.; Fagot, D.; Glab, N.; Goudeau, H.; Goudeau, M.; Guerrier, P.; Houghton, P.; Hendriks, H.; Kloareg, B.; Lippai, M.; Marie, D.; Maro, B.; Meijer, L.; Mester, J.; Mulner-Lorillon, O.; Poulet, S.; Schierenberg, E.; Schutte, B.; Vaulot, D.; Verlhac, M. *Biol. Cell* **1995**, *83*, 105; (c) Havlicek, L.; Hanus, J.; Vesely, J.; Leclerc, S.; Meijer, L.; Shaw, G.; Strand, M. *J. Med. Chem.* **1997**, *40*, 408.
4. (a) Kaur, G.; Stetler-Stevenson, M.; Sebers, S.; Worland, P.; Sedlacek, H.; Myers, C.; Czeck, J.; Naik, R.; Sausville, E. *J. Nat. Cancer Inst.* **1992**, *84*, 1736; (b) Carlson, B.; Dubay, M. M.; Sausville, E. A.; Brizuela, L.; Worland, P. J. *Cancer Res.* **1996**, *56*, 2973; (c) Sedlacek, H. H.; Czech, J.; Naik, R.; Kaur, G.; Worland, P.; Losiewicz, M.; Parker, B.; Carlson, B.; Smith, A.; Senderowicz, A.; Sausville, E. *Int. J. Oncol.* **1996**, *9*, 1143; (d) Senderowicz, A. M. *Invest. New Drugs* **1999**, *17*, 313; (e) Stadler, W. M.; Vogelzang, J.; Amato, R.; Sosman, J.; Taber, D.; Liebowitz, D.; Vokes, E. E. *J. Clin. Oncol.* **2000**, *18*, 371; (f) Zhai, S.; Senderowicz, A. M.; Sausville, E. A.; Figg, W. D. *Ann. Pharmacother.* **2002**, *36*, 905.
5. (a) Misra, R. N.; Xiao, H. Y.; Kim, K. S.; Lu, S.; Han, W. C.; Barbosa, S. A.; Hunt, J. T.; Rawlins, D. B.; Shan, W.; Ahmed, S. Z.; Qian, L.; Chen, B. C.; Zhao, R.; Bednarz, M. S.; Kellar, K. A.; Mulheron, J. G.; Barorsky, R.; Roongta, U.; Kamath, A.; Marathe, P.; Ranadive, S. A.; Sack, J. S.; Tokarski, J. S.; Pavletich, N. P.; Lee, F. Y.; Webster, K. R.; Kimball, S. D. *J. Med. Chem.* **2004**, *47*, 1719; (b) Kim, K. S.; Kimball, S. D.; Misra, R. N.; Rawlins, D. B.; Hunt, J. T.; Xiao, H.-Y.; Lu, S.; Qian, L.; Han, W.-C.; Shan, W.; Mitt, T.; Cai, Z.-W.; Poss, M. A.; Zhu, H.; Sack, J. S.; Tokarski, J. S.; Chang, C.-Y.; Pavletich, N.; Kamath, A.; Humphreys, W. G.;

Marathe, P.; Bursuker, I.; Kellar, K. A.; Roongta, U.; Batorsky, R.; Mulheron, J. G.; Bol, D.; Fairchild, C. R.; Lee, F. Y.; Webster, K. R. *J. Med. Chem.* **2002**, *45*, 3905.
6. Synthetic compounds were characterized by HPLC, 400 or 500 MHz ^{1}H NMR, and LC/MS.
7. (a) Doyle, M. P.; Siegfried, B.; Dellaria, J. F. *J. Org. Chem.* **1977**, *42*, 2426; (b) Hodgetts, K. J.; Kershaw, M. T. *Org. Lett.* **2002**, *4*, 1363.
8. Prepared by addition of 5-*tert*-butyl-2-chloromethyl-oxazole[5a] to a solution of thioacetic acid (2 equiv) and DIPEA (2 equiv) in THF at room temperature. The reaction mixture was stirred for 0.5 h, concentrated in vacuo then filtered through silica gel and eluted with diethyl ether. The crude material, which discolored upon concentration was obtained in quantitative yield and used in this form.
9. Abdel-Magid, A. F.; Carson, A. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.
10. In solving the X-ray structure of **7** complexed to CDK2 it was found that the nitrogen in the pyridine ring could be placed in two alternative positions because the electron density was indiscriminate. The pyridine nitrogen could (1) face the hinge region or (2) be oriented on the same side as the sulfur of the thiazole ring. Energy calculations of the different orientations of the pyridine ring reveal that the orientation of the nitrogen on the same side as the sulfur is $\sim$2 kcal lower in energy than the alternative orientation with the pyridine and thiazole rings being near planar. The starting coordinates of the structures were generated using CONCORD™. The evaluated conformations were subjected to 30 steps of DFT energy optimizations as implemented in Jaguar (Jaguar 4.0, Schrodinger, Inc., Portland, OR, 1991–2000) at B3LYP/G-31G** and a single-point energy was calculated for the resulting geometries using B3LYP/cc-PVTZ(-f)$^+$.