Indian Journal of Chemistry
Vol. 59A, September 2020, pp. 1354-1358

Tetrandrine derivatives IVa-IVd: Structural analysis and their inhibition rate against protein tyrosine kinase, and HL60 & A549 cancer cell lines

Chen Chen[a] & Ye Lu[b]*

[a]Department of Geriatrics; [b]Department of Endocrinology, Suzhou Ninth People's Hospital, Jiang su Suzhou 215200, China

*E-mail: baocizhao737@163.com

Received 02 May 2020; accepted 29 July 2020

Four tetrandrine (TET) derivatives (IVa-IVd) have been synthesized by Suzuki reaction of boronic acid derivatives and 5-bromo-tetrandrine obtained by bromination using tetrandrine as the starting material. The structures are analyzed by ^{1}H NMR, ^{13}C NMR and ESI-MS. The inhibition activities of IVa-IVd against HL60 and A549 cancer cell lines have been investigated by CCK-8 assay. The compounds having significant (P <0.040) inhibition activities are secondarily screened by MTT assay. The inhibitory activities of IVa-IVd against protein tyrosine kinases (PTKs) are tested by ELISA. The results show that IVa, IVb and IVc exhibit significant inhibition activities against the two cancer cell lines. The IVb and IVc show highly significant (P <0.001) inhibition activities against fibroblast growth factor receptor 1 (FGFR1).

Keywords: Anticancer, Bromination, CCK-8, FGFR1, PTKs, Suzuki reaction, Tetrandrine

Tetrandrine (TET) is isolated from the roots of *Stephania tetrandra* (S. Moore) (Menispermaceae) which is commonly used in traditional Chinese medicine as an antirheumatic, anti-inflammatory and antihypertensive agent. As the main component of the alkaloid extracted from the root tuber of *S. tetrandra* and *S. japonica* (Japanese stephania), TET has a wide range of pharmacological effects viz. antiplatelet aggregation, Ca^{2+} channel block, immunosuppressive and free radical scavenging effects[1]. In recent years, its antitumor effect has attracted much attention[2]. Tetrandrine has diverse anti-inflammatory, immuno-suppressive and cytoprotective effects[3]. It is the lead compound and its structure is modified by protein tyrosine kinase (PTKs) family plays an important role in cell growth, proliferation and differentiation. However, gene mutation, gene fusion and other pathological mechanisms can lead to the continuous activation of PTKs, abnormal cell regulatory function and tumor induction[4].

TET has been used as an antifibrotic drug to treat the lesions of silicosis in China since the 1960s. It is relatively non-toxic to humans, even at the administration of 240 mg, intramuscularly (i.m.) thrice daily. TET exhibited stronger activity to reverse drug resistance to daunorubicin, vinblastine and doxorubicin (DOX) in leukemia cells[5]. The c-Met is the prototype member of a subfamily of RTKs, which includes Ron, which is structurally distinct from other RTK families, c-Met signaling is implicated in a wide variety of human malignancies in various cancers[6]. Under normal conditions, the balance is maintained by the antagonistic regulation of tyrosine kinase and tyrosine phosphatase[7]. It is a research hotspot of antitumor drugs to block the signal transmission of tumor cells by tyrosine kinase to achieve the purpose of tumor treatment.

Materials and Methods

Instruments and reagents

ZF type three purpose ultraviolet analyzer; Advance-500MHz superconducting NMR ($CDCl_3$ as a solvent, TMS as internal standard); Agilent 1100sl type ion trap mass spectrometer; molecular device SPECTROMAX 190 type enzyme labelling instrument; smart cell-CO_2 type incubator and SW-2f type ultra-clean workbench were used in this study. Human lung cancer cell line (A549), and human promyelocytic leukemia cell line (HL60) were purchased from Sigma-Aldrich Co.; while as DMEM/ high glucose medium, 0.25% trypsin solution and protein tyrosine kinase (PTKs) were procured from Shanghai Institute of Medicine. All reagents used were of analytical grade.

Synthesis

The bromination of tetrandrine was carried out in the presence of trifluoroacetic acid and stereospecific cross-coupling on brominated alkene with palladium-

catalyst as shown in Scheme 1[8]. Four new phenyl substituted tetrandrine were synthesized.

Synthesis of compound II

A volume of 0.5 mL of **I**, 10 mL of trifluoroacetic acid, and 5 mL of water were added in a single neck bottle, stirred to dissolve them; slowly injected with 0.6 mL bromine, acetic acid (2.5 mL) solution, and made them to react in an ice salt bath for 2.5 h. For quenching, 25 mL of ice water was added. Ammonia water was added for maintaining acidic nature of product. Product was extracted using dichloromethane (2×100 mL), and combined with the organic phase, washed with saturated salt water (3×25 mL), dried with anhydrous sodium sulfate, and the solvent was evaporate under reduced pressure. The residue was re-crystallized with ether and purified by silica gel column chromatography (eluent: V (methanol): V (ethyl acetate): 7:3). The yield of white amorphous powder was 88%.

Synthesis of IVa-IVd

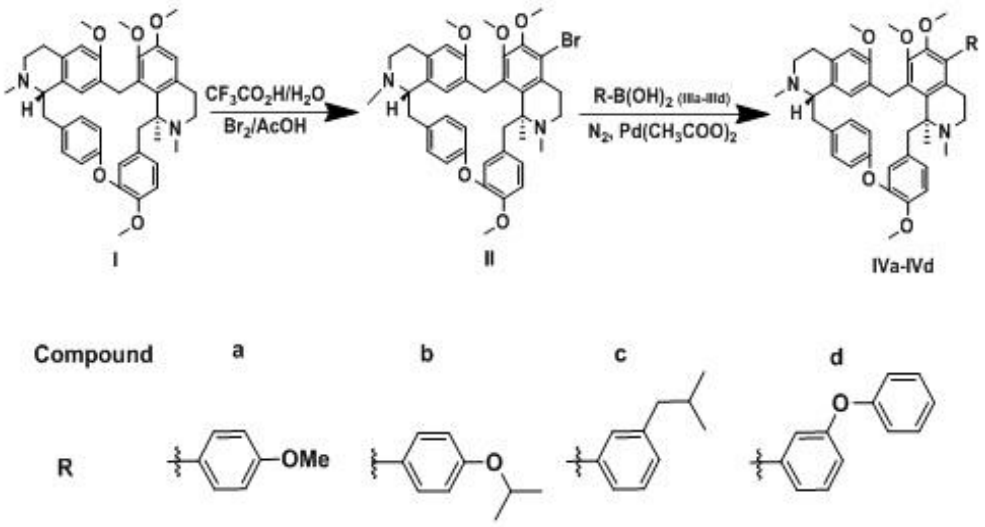
About 135 mg (0.1 mmol), 10 mL toluene and 2.5 mL of water were added to the three-necked flask, and then stirred to dissolve the contents. About 1.0 mol of potassium acetate (AcOK) was added, and then vacuumed and charged with nitrogen for many times in 20℃ ultrasonic degassing for 20 min. Pd $(CH_3COO)_2$ 110 mg and 4-biphenyl-phenyl boric acid **(**IIIa**)** 30.1 mg (0.2 mmol) were added, and the reaction was carried out under the condition of reflux (90℃) protected by nitrogen for 8 h. Contents were cooled to room temperature (37℃), added with 3 mL distilled water for quenching reaction. After filtration, 15 mL ethyl acetate were added to the filtrate, washed with saturated salt water (3×50 mL), dry it with anhydrous sodium sulfate, evaporate and remove the solvent under reduced pressure, and purify 5-(4-bi-phenyl) tetrandrine **(**IVa**)** by TLC [developer: V (dichloromethane): V (methanol): V (ethyl acetate): V (petroleum ether) = 15:1:1:1]

Determination of biological activity

Cell Counting Kit-8 (CCK-8), sensitive colorimetric assay was used to determination of cell viability in cell proliferation and cytotoxicity assays. The cell density was adjusted to 5.0×10^4 cells/mL after the cells were digested with 0.25% trypsin. Each cell suspension was inoculated with 100 μL of cell culture into 96-well plate. The cells were cultured in an incubator with 5% CO_2 at 36℃ for 12 h. Each well was added with different concentrations of drugs, each group had three wells. The control group was added with the same volume of medium as the volume of drug added to other wells. All groups were cultured in an incubator containing 5% CO_2 at 36℃ for 72 h before the termination of culture growth. Each well was added with 10 μL LCC-8 solution, mixed well, and incubated in the cell incubator for 1 h to detect the OD value of each well at the wavelength of 490 nm. The cell survival rate was calculated by given equation:

$$\text{Cell survival rate\%} = \frac{\text{OD experiment}}{\text{OD control group}} \times 100$$

The survival rate of the control group was recorded as 100%.



Scheme 1 — Schematic for the bromination of tetrandrine for synthesis of the desired compounds (Iva-IVd)

Cell culture

HL60 and A549 cells were inoculated in 1640 medium containing 10% fetal bovine serum, 100 μg/mL penicillin, and 100 μg/mL streptomycin and cultured in 5% CO_2 incubator at 36℃. Logarithmic growth phase cells were used.

MTT active re-screening was done when the concentration was 10 μmoL/mL, i.e., the compounds with inhibition rates above 50% were screened by the MTT method. About 5 mg/mL MTT 20L was added into each well and kept in the incubator for 3-4 h. Added 100 μL solution into each hole, kept the solution in the incubator overnight, and made the crystal fully dissolved, and measured the absorption at 570 nm. The IC_{50} of each tumor cell was calculated by the software.

Determination of PTK inhibitory activity

The reaction substrate poly (Glu, Tyr) 4:1 was added to 2.0 μg/mL-1125 μL well-coated enzyme plate was diluted by PBS enzyme without K, and the reaction was carried out at 36℃ for 12 h. Discarded the liquid from the wells and washed the plate with t-PBS [including 0.1% Tween-20 PBS without K^+, 200 μL/well] thrice, each time for 5 min. Dried the enzyme plate at 36℃ for 90 min and added 49 μL ATP solution diluted by reaction buffer [50 mmol/mL-HEPES pH 7.4, 50 mmol/mL-MgCl, 0.5 mmol/mL-MnCl, 0.2 mmol mL Na_3VO_4, 1 mmol/mL-DTT], added 1.0 μL diluted solution of each compound to be tested in each well, and then add 50 μL CMET, ALK, FGFR1, RET, EGFR, ErbB2 kinase domain recombinant protein diluted by reaction buffer to start the reaction. Each experiment was to set with two wells without the ATP control well. The reaction was carried out in a shaker (100rpm) at 36℃ for 1 h. The liquid was discarded from the well and the plate was washed with t-PBS thrice and added with 100 μL of py99 diluent (the antibody was diluted with 5 mg/mL t-PBS 1:500) in each well, and left to react for 0.5 h at 36℃. Later discarded the liquid from the wells and washed the plate with t-PBS for thrice and added horseradish peroxidase-labeled sheep anti-mouse second antibody diluent (the antibody was diluted with t-pbs1: 2000 containing 5 mg/ mL), 100 μL /well, and left to react in a shaker at 36℃ for 0.5 h. Discarded the liquid and washed the plate with t-PBS thrice. Added 100 μL /well of OPD colour developing solution of 2 mg/mL [dilute with 0.1 mmol/mL-citric acid/sodium citrate buffer (pH 5.4) containing 0.03% H_2O_2, and stopped the reaction at 25℃ for 1-10 min in dark/light, added 2 mol/mL H_2SO_4 well to stop the reaction, read with the enzyme scale. The OD value of each sample was determined at 490 nm. The IC_{50} was obtained by a four-parameter regression method with software attached to the enzyme labelling instrument using equation:

$$\text{Inhibition rate (\%)} = 1 - \frac{\text{OD compound without control}}{\text{OD negative } vs. \text{ OD without ATP control}} \times 100$$

Results and Discussion

Palladium-catalyzed Suzuki reaction is an important reaction for the construction of C-C bond compounds. It has the advantages for wide range of substrates, offers mild reaction conditions, and good regioselectivity. With the help of the Suzuki reaction, scientists have analysed hydratoxin and natural products of polyketones, diopside. In this paper, four new tetrandrine derivatives **(IVa-IVd)** were synthesized by Suzuki reaction after the 5-selective bromination of **I** and the structures were analysed by various analytical methods including ^{1}H NMR, ^{13}C NMR and ESI-MS. Among them, the synthesis of II is the key step of this experiment. When the drop acceleration of bromine is too fast, the temperature of the system exceeds −15℃ due to the exothermic reaction process, and more impurities are produced. By adjusting the drop acceleration of bromine, keeping the reaction temperature below −15℃, the impurities are reduced, and the yield is 94%, which is suitable for large-scale preparation. Suzuki reaction requires strict deaeration, and the presence of oxygen will reduce the yield. The whole process is carried out in a nitrogen atmosphere. After treatment, thin layer chromatography was used. Due to the similar polarity of IV and II, the second expansion was carried out during the experiment, which made the RF value of **IV** and **II** different greatly and separated.

Analysis of structural data

5-(4-methoxyphenyl) tetrandrine (IVa)

Light yellow solid; yield 56%; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.37 (dd, J = 8.1 Hz, 1H), 7.17 (dd, J = 8.3 Hz, 1H), 6.93 (s, 1H), 6.89 (d, J = 3.5 Hz, 1H), 6.83 (dd, J = 7.9 Hz, 1H), 6.61 (d, J = 1.4 Hz, 1H), 6.58 (d, J = 2.3 Hz, 1H), 6.56–6.52 (m, 3H), 6.33 (dd, J = 8.2, 2.1 Hz, 1H), 6.04 (s, 1H), 3.87(s, 3H), 3.73 (s, 3H), 3.67 (s, 1H), 3.49 (s, 1H), 3.47 (s, 3H), 3.45 (s, 1H), 3.41 (s, 3H), 3.37 (s, 1H), 3.31 (s, 1H), 3.24 (s, 3H), 3.07–2.91 (m, 3H), 2.88–2.73 (m, 5H), 2.68 (s, 1H), 2.67 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 161.22, 159.03, 154.75, 150.36, 149.40,

148.64, 148.10, 145.06, 142.65, 136.07, 133.61, 132.81, 132.42, 131.47, 129.22, 128.93, 128.80, 126.87, 123.88, 122.90, 121.52, 121.07, 119.23, 117.13, 112.34, 113.93, 112.67, 105.25, 100.02, 99.60, 64.75, 61.36, 61.10, 57.12, 56.78, 56.35, 46.25, 43.63, 43.54, 43.03, 39.06, 32.96, 30.73, 26.27, 23.72. HRMS (ESI): $[M+H]^+$Calcd for $C_{47}H_{52}N_2O_6$ 740.3825, found: 740.3817.

5-(4-isopropoxyphenyl) tetrandrine (IVa)

Light yellow solid; yield 54%; 7.34 (dd, J = 7.76 Hz, 1H), 7.12 (dd, J = 7.27 Hz, 1H), 6.95 (s, 1H), 6.87 (d, J = 3.7Hz, 1H), 6.82 (dd, J = 7.6 Hz, 1H), 6.56 (d, J = 2.1 Hz, 1H), 6.54 (d, J = 1.9 Hz, 1H), 6.53–6.49 (m, 3H), 6.31 (dd, J = 8.3, 2.1 Hz, 1H), 6.10 (s, 1H), 4.96 (s, 1H), 3.82 (s, 3H), 3.68 (s, 1H), 3.51 (s, 1H), 3.49 (s, 3H), 3.47 (s, 1H), 3.43 (s, 3H), 3.39 (s, 1H), 3.33 (s, 1H), 3.25 (s, 3H), 3.08–2.96 (m, 3H), 2.91–2.77 (m, 5H), 2.71 (s, 1H), 2.68 (s, 3H), 2.29 (s, 3H) 1.34(m,6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 161.19, 158.94, 154.87, 150.38, 149.47, 148.65, 148.27, 144.95, 142.67, 135.98, 133.66, 133.01, 132.46, 131.49, 129.28, 129.03, 128.60, 126.89, 123.89, 122.86, 121.48, 121.11, 119.19, 117.14, 112.31, 113.96, 112.69, 105.21, 100.12, 99.63, 73.45, 64.77, 61.39, 61.16, 57.17, 56.81, 56.39, 46.29, 43.66, 43.52, 43.12, 38.79, 32.87, 30.76, 26.28, 23.76, 22.93. HRMS (ESI): $[M+H]^+$Calcd for $C_{49}H_{56}N_2O_6$ 768.4138, found: 768.4132.

5-(4-isobutylphenyl) tetrandrine (IVc)

Light yellow solid; yield 55%; Light yellow solid; yield 56%; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.35 (dd, J = 7.91 Hz, 1H), 7.15 (dd, J = 7.6 Hz, 1H), 6.94 (s, 1H), 6.85 (d, J = 3.5 Hz, 1H), 6.81 (dd, J = 7.9 Hz, 1H), 6.67 (d, J = 2.4 Hz, 1H), 6.61 (d, J = 2.7 Hz, 1H), 6.57–6.51 (m, 3H), 6.37 (dd, J = 8.4 Hz, 1H), 6.07 (s, 1H), 3.80 (s, 3H), 3.75 (s, 1H), 3.50 (s, 1H), 3.45 (s, 3H), 3.43 (s, 1H), 3.40 (s, 3H), 3.36 (s, 1H), 3.33 (s, 1H), 3.27 (s, 3H), 3.09–2.98 (m, 3H), 2.82–2.73 (m, 5H), 2.73 (m, 1H), 2.69 (s, 1H), 2.65 (s, 3H),2.30 (s, 3H), 1.97 (m, 1H), 1.12 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 161.20, 159.21, 154.64, 150.47, 149.48, 148.54, 148.17, 145.13, 142.71, 136.12, 133.66, 132.61, 132.47, 131.42, 129.92, 128.23, 128.87, 126.80, 123.86, 122.80, 121.57, 121.15, 119.31, 117.24, 112.37, 113.73, 112.69, 105.55, 100.62, 99.23, 61.39, 61.17, 57.06, 56.38, 56.05, 49.65, 46.27, 43.63, 43.56, 43.23, 39.17, 33.04, 32.10, 30.93, 25.62, 26.27, 23.72. HRMS (ESI): $[M+H]^+$Calcd for $C_{50}H_{58}N_2O_5$ 766.4346, found: 766.4361.

5-(4-phenoxyphenyl) tetrandrine (IVa)

Light yellow solid; yield 54%; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.36 (dd, J = 6.7 Hz, 1H), 7.14 (dd, J = 8.3 Hz, 1H), 6.83 (s, 1H), 6.77 (d, J = 3.5 Hz, 1H), 6.71 (dd, J = 7.9 Hz, 1H), 6.63 (d, J = 1.4 Hz, 1H), 6.60-6.57 (m, 6H), 6.55–6.50 (m, 3H), 6.34 (dd, J = 5.2Hz, 1H), 6.14 (s, 1H), 3.90(s, 3H), 3.79 (s, 3H), 3.71 (s, 1H), 3.63 (s, 1H), 3.54 (s, 3H), 3.49 (s, 1H), 3.43 (s, 3H), 3.39 (s, 1H), 3.36 (s, 1H), 3.32 (s, 3H), 3.16–3.03 (m, 3H), 2.93–2.78 (m, 5H), 2.73 (s, 1H), 2.69 (s, 3H), 2.27 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 160.82, 160.03, 155.25, 151.56, 149.90, 148.84, 148.09, 145.26, 142.35, 136.27, 133.57, 132.63, 132.22, 131.97, 129.62, 128.33, 128.67, 126.53, 123.67, 122.47, 121.63, 121.17, 119.29, 117.25, 112.43, 113.63, 112.36, 105.25, 100.06, 99.32, 64.61, 61.70, 61.22, 57.31, 56.87, 56.15, 46.49, 43.33, 43.24, 42.83, 39.25, 32.66, 31.23, 27.17, 22.12. HRMS (ESI): $[M+H]^+$Calcd for $C_{52}H_{54}N_2O_6$802.3982, found: 802.3998.

Biological Activity

The inhibitory activities of IVa-IVdon HL60 cells and A549 cells are shown in (Fig. 1). It can be seen from figure 1 that compound IVa, IVb, and IVc have some significant (P <0.040) inhibitory activities. The inhibitory activity of IVa-IVd on protein tyrosine kinase is shown in Fig. 2. It can be seen from Fig. 2 that the inhibitory activity of IVb and IVc on receptor tyrosine kinase FGFR1 is more than 47%, and the activity is higher than tetrandrine to some extent. In conclusion, IVa and IVb not only have some inhibitory activity on HL60 and A549 cells but also have high selectivity for tyrosine kinase FGFR1. It can be further modified to find more active compounds.

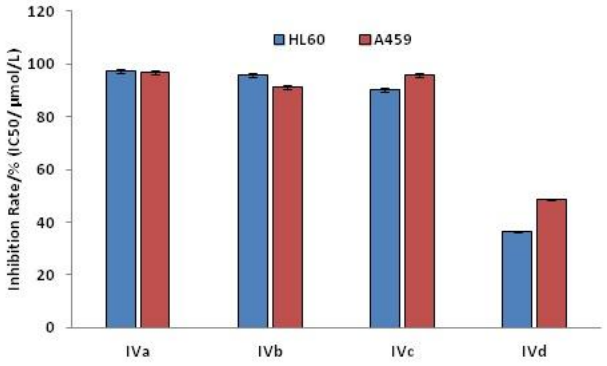


Fig. 1 — Inhibition activities of IVa-IVd against HL60 and A549 human cancer cell lines

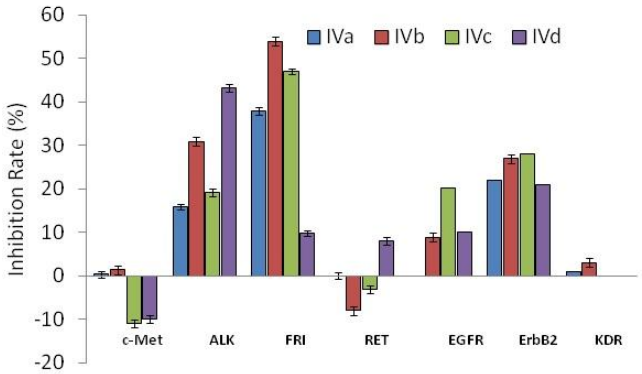


Fig. 2 — Inhibition ratio of VIa-IVd against different PTKs

Conclusions

Four new derivatives of tetrandrine were synthesized and analyzed for their structural details. The preliminary antitumor activity showed that IVa, IVb, and IVc had some significant (P <0.040) inhibitory activity on HL60 cells and A549 cells. Among them, IVa and IVb have highly significant (P <0.001) inhibitory activity on receptor tyrosine kinase FGFR1, which can be further modified as potential drugs. On the basis of this study, the antitumor activity, structure-activity relationship, and mechanism of action of other compounds of the same series are in progress in order to obtain more effective antitumor drugs.

Conflict of interest

The authors declare no conflict of interests in this study.

References

1 Kuo PL & Lin CC, *Life Sci*, 73 (2003) 243.
2 Xiao-Hui C, Shuai W & Bao-An C, *Chinese J Nat Med*, 9 (2011) 473.
3 Wu JM, Chen Y, Chen JC, Lin TY & Tseng SH, *Cancer Lett*, 287 (2010) 187.
4 Fan Rui, Ban Shu-rong, Fang Lian-hua & Li Qing-shan, *Chinese J Med Chem*, 22 (2011) 178.
5 Xu WL, Shen HL, Ao ZF, Chen BA, Xia W, Gao F & Zhang YN, *Leukemia Res*, 30 (2006) 407.
6 Jung KH, Park BH & Hong SS, *Arch Pharm Res*, 35 (2012) 595.
7 Wiriyachitra P & Cava MP, *J Org Chem*, 42 (1977) 2274.
8 Miyaura N, Yamada K & Suzuki A, *Tetrahedron Lett*, 20 (1979) 3437.