A facile approach to 2-alkoxyindolin-3-one and its application to the synthesis of \( N \)-benzyl matemone†

Makoto Shimizu, Hayao Imazato, Isao Mizota and Yusong Zhu

2-Alkoxycarbonylindolin-3-one is synthesized from a methoxyglycine derivative via a 1,2-aza-Brook rearrangement followed by cyclization with bis(trimethylsilyl)aluminum chloride. A short-step synthesis of \( N \)-benzyl matemone is successfully carried out using the present indolin-3-one synthesis.

Introduction

Heterocyclic compounds possessing an oxindole skeleton have received considerable attention due to the widespread existence of naturally occurring bioactive materials containing this particular heterocycle. Among them bromine-containing and/or 2-alkoxy indolin-3-one and indole alkaloids such as matemone, cephaline, and bromoaplysinopisin show intriguing bioactivities. Regarding matemone, it was isolated from the Indian Ocean sponge Iotrochota purpurea and its structure was elucidated in 2000. Matemone shows mild cytotoxicity against three cancer cell lines and marginal antibacterial activity against Staphylococcus aureus. We have been interested in the reactivity of \( \alpha \)-iminoesters in umpolung reactions, and a facile indolin-3-one synthesis via aza-Brook rearrangement has been developed (Scheme 1).

However, difficulties have been encountered regarding the substituents at the 2-position, i.e., only 2,2-disubstituted derivatives could be synthesized by our previously reported procedure (compound 2, \( R = A r \) or \( C O_2 R' \)).

For the construction of matemone and related structures, a procedure using the aldimine of type 1 (\( R = H \)) is needed; in particular, a facile approach to 2-mono-substituted indolin-3-one, a key intermediate is needed. We have now found that methoxyglycine derivative 11 could be isolated in good yield and served as a stable imine precursor.\(^\dagger\) Cyclization reaction of this methoxy amino diester 11 was carried out with \((\text{TMS})_2\text{AlCl}\), and the results are summarized in Table 1.

Results and discussion

For the synthesis of this particular aldimine 10, we examined several approaches, such as direct imination of glyoxylate through dehydration and oxidation of glycine derivatives (\( \text{MnO}_2, \text{DDQ}, \text{NBS}, \text{etc.} \)).\(^7\) However, none of the attempted procedures worked, and only complex mixtures were obtained (Scheme 3).

We finally found that the methoxyglycine derivative 11 could be isolated in good yield and served as a stable imine precursor.\(^\dagger\) Cyclization reaction of this methoxy amino diester 11 was carried out with \((\text{TMS})_2\text{AlCl}\), and the results are summarized in Table 1.

An initial examination using 2.0 equiv. of \((\text{TMS})_2\text{AlCl}\) in \( \text{EtCN} \) as a solvent led to the formation of the desired indolin-3-one 12 in only 15% yield (entry 1). Increasing the amount of \((\text{TMS})_2\text{AlCl}\) to 4.0 equiv. improved the yield to 56% (entry 2). However, the use of a large excess of the reagent decreased the yield (entry 3). Use of other solvents such as \( \text{CH}_2\text{Cl}_2, \text{Et}_2\text{O}, \) and \( \text{THF} \) was unsuccessful (entries 7, 9 and 10). Regarding the reaction temperature, the treatment of the starting
material 11 with (TMS)$_2$AlCl at $-78^\circ$C, followed by warming the whole mixture to room temperature recorded the best result (entry 2). The following Scheme 4 shows a possible reaction pathway.

First, the aldimine 10 is formed \textit{in situ} by the treatment of the methoxyglycine derivative 11 with bis(trimethylsilyl) aluminum chloride. The formation of the imine 10 was detected by a direct injection EI-MS (m/z 313). This imine 10 would be attacked by the second equivalent of bis(trimethylsilyl)aluminum chloride to form the aluminum enolate 13 via an aza-Brook rearrangement. A subsequent Dieckmann cyclization followed by hydrolysis gives the indolin-3-one 12 (Scheme 5).

For the synthesis of matemone 3, the introduction of the methoxy group at the C-2 position is needed. After several attempts using a series of oxidation reagents, we found that the oxidation of the silyl enol ether 15 with NBS in methanol gave satisfactory results.\textsuperscript{11,12} However, selective reduction at the ester moiety was not successful.\textsuperscript{13} Bis-reduction at the ketone and the ester moieties followed by oxidation at the benzylic alcohol was also failed to give only complex mixtures. We then changed the order of the functional group transformations, \textit{i.e.}, reduction of the ester moiety, followed by the introduction of the methoxy group. This procedure worked well to give N-benzyl matemone 17 in high yield (Scheme 6).

This intriguing oxidation into the methoxy derivative 17 is explicable in terms of the formation of the iminium species 19, which is attacked by methanol (Scheme 7).

We next attempted removal of the benzyl group under a series of conditions (RSH/base, TMSI, Ca or Na/liq. NH$_3$, H$_2$/Pd or Pt, \textit{etc.}). Although a small amount of matemone was detected by the mass spectra of the crude reaction mixtures, attempted isolation by silica gel chromatography was not

### Table 1: Preparation of indolin-2-one 12

| Entry | Temperature       | (TMS)$_2$AlCl (equiv.) | Solvent   | Yield of 12\textsuperscript{a} (%) |
|-------|------------------|------------------------|-----------|-----------------------------------|
| 1     | $-78^\circ$C to rt | 2.0                    | EtCN      | 15                                |
| 2     | $-78^\circ$C to rt | 4.0                    | EtCN      | 56                                |
| 3     | $-78^\circ$C to rt | 6.0                    | EtCN      | 38                                |
| 4     | $-40^\circ$C to rt | 4.0                    | EtCN      | 37                                |
| 5     | $-78^\circ$C to 0$^\circ$C | 4.0            | EtCN      | 36                                |
| 6     | $-78^\circ$C to 50$^\circ$C | 4.0            | EtCN      | 46                                |
| 7     | $-78^\circ$C to rt | 4.0                    | CH$_2$Cl$_2$ | 6                                |
| 8     | $-78^\circ$C to rt | 4.0                    | EtCN/CH$_2$Cl$_2$ (1 : 1) | 50                                |
| 9     | $-78^\circ$C to rt | 4.0                    | Et$_2$O   | 26                                |
| 10    | $-78^\circ$C to rt | 4.0                    | THF       | 38                                |

\textsuperscript{a} Isolated yield.
successful. We also attempted the isolation as an acetate form by treatment of the whole reaction mixtures with an excess AcCl/base. However, the acetate was not isolated in sufficient quantity. Studies indicated that unprotected matemone was unstable due to a solvent-induced polymerization process. Therefore, matemone was immediately converted to the stable acetate derivative 3 \((R^2 = \text{Ac})\), and detailed spectroscopic analyses were carried out with the acetate derivative. We found that \(N\)-protected matemone 18 was also reasonably stable and would be subject to further functional group interconversions.

**Scheme 4** A proposed reaction mechanism of the indolin-3-one 12 synthesis.

**Scheme 5** Introduction of 2-methoxy group.

**Conclusions**

We have found that the methoxyglycine derivative 11 is a good precursor to the aldimine 10 derived from glyoxylate, and the subsequent treatment of this particular methoxyglycine 11 with bis(trimethylsilyl)aluminum chloride provides 2-alkoxycarbonylindolin-3-ones. Further oxidation of the silyl enol ether prepared from the 2-alkoxycarbonylindolin-3-one undergoes a facile oxidation reaction with NBS in methanol to give the 2-methoxy derivatives in high yields. This procedure has proved to be effective for the synthesis of \(N\)-benzyl matemone as a reasonably stable derivative. Although we have not examined the bioactivity of the \(N\)-benzyl matemone 17 yet, we will submit it and other derivatives to bioassay in due course.
Experimental

General aspects

Infrared spectra were determined on a JASCO FT/IR-460 plus spectrometer. 1H NMR and 13C NMR spectra were recorded with a JEOL ECX-400P, or a JEOL A-500 spectrometer using tetramethylsilane as an internal standard. Mass spectra were recorded on a JEOL MS-70D spectrometer. Propionitrile (EtCN) and acetonitrile (MeCN) were distilled from phosphorus pentoxide and then from calcium hydride and stored over Molecular Sieves 4 Å. Dichloromethane (CH2Cl2) was distilled from calcium hydride and stored over Molecular Sieves 4 Å. Toluene was dried over calcium chloride, distilled, and stored over Molecular Sieves 4 Å. Diethyl ether (Et2O) and tetrahydrofuran (THF) were distilled from benzophenone ketyl immediately before use or purified by glass column organic solvent purification system of Nikko Hanssen & Co., Ltd. MeOH was heated at reflux over magnesium for 5 h, distilled, and stored over Molecular Sieves 3 Å. Purification of products was performed by column chromatography on silica gel (Kanto Silica Gel 60N) and/or preparative TLC on silica gel (Merck Kiesel Gel GF254 or Wako Gel B-5F).

Methyl 4-bromo-2-[(2-ethoxy-1-methoxy-2-oxoethyl)amino]-benzoate (11)

In a 30 mL two-necked round-bottomed flask equipped with a magnetic stirring bar, a rubber septum and an argon balloon were placed methyl 2-amino-4-bromobenzoate (460.0 mg, 2.00 mmol) prepared according to the reported procedures,16 ethyl glyoxylate (1.23 mL, 6.00 mmol, 50% in toluene), and methanol (10.0 mL), respectively. The mixture was stirred at reflux for 16 h. After cooling to room temperature, the mixture was concentrated in vacuo to give a crude oil, which was purified by silica gel chromatography (hexane : ethyl acetate = 6 : 1) to give the title compound 11 (604.6 mg, 87%) as white crystals.

Yield 87% (604.6 mg); white crystals; mp 86–88 ºC; Rf = 0.50 (hexane : ethyl acetate = 4 : 1); 1H NMR (400 MHz, CDCl3) δ 1.36 (t, J = 7.3 Hz, 3H), 3.30 (s, 3H), 3.89 (s, 3H), 4.34 (q, J = 7.3 Hz, 2H), 5.26 (d, J = 6.4, 1H), 6.88–6.91 (m, 1H), 7.13 (d, J = 1.8 Hz, 1H), 7.79 (d, J = 8.7 Hz, 1H), 8.98 (d, J = 6.4 Hz, 1H); 13C NMR (100 MHz, CDCl3) δ 14.1, 51.6, 51.9, 62.2, 81.4, 110.9, 116.1, 120.7, 129.5, 132.7, 146.8, 167.9, 168.0; IR (neat) 3432, 2952, 1743, 1693, 1571, 1505, 1240, 1095, 1064, 769 cm⁻¹; HRMS (EI) calcd for C17H16BrNO3Si (M⁺) 382.0974 found 382.0970.

General procedure: synthesis of ethyl 6-bromo-3-hydroxy-1H-indole-2-carboxylate (12) (Table 1)

Under an argon atmosphere, a solution of methyl 4-bromo-2-[(2-ethoxy-1-methoxy-2-oxoethyl)amino]-benzoate 11 (100.0 mg, 0.29 mmol) in EtCN (30.0 mL) was placed at –78 ºC and to it was added a propionitrile solution (10 mL) of [TMS]2AlCl, which was prepared by mixing aluminum chloride (52.0 mg, 0.39 mmol) and (TMS)3Al-Et2O (0.62 mL, 0.77 mmol, 1.25 M in Et2O) at room temperature in another flask. After the mixture was stirred for 2 hours at room temperature, to it was added saturated aqueous potassium fluoride followed by a saturated aqueous Rochelle’s salt to quench the reaction. The whole mixture was extracted with ethyl acetate (10 mL × 3). The combined organic phases were washed with brine, dried over Na2SO4, and concentrated in vacuo to give a crude product. Purification by silica gel column chromatography (hexane : ethyl acetate = 4 : 1 as an eluent) gave ethyl 6-bromo-3-hydroxy-1H-indole-2-carboxylate 12 (44.8 mg, 56%) as yellow crystals.

Yield 56% (44.8 mg); mp 167–169 ºC; yellow crystals; Rf = 0.32 (hexane : ethyl acetate = 4 : 1); 1H NMR (400 MHz, CDCl3) δ 1.41 (t, J = 6.9 Hz, 3H), 4.39 (q, J = 6.9 Hz, 2H), 7.04–7.06 (m, 1H), 7.48–7.49 (m, 1H), 7.58–7.60 (m, 1H), 8.79 (s, 1H), 10.70 (s, 1H); 13C NMR (100 MHz, CDCl3) δ 13.2, 58.7, 108.1, 113.6, 115.5, 118.0, 119.9, 120.3, 132.4, 142.8, 160.9; IR (neat) 3341, 1672, 1608, 1583, 1308, 1240, 1114, 1018, 770 cm⁻¹; HRMS (EI) calcd for C11H10BrNO3 (M⁺) 282.9844 found 282.9842.

Ethyl 6-bromo-3-[[tert-butyldimethylsilyloxy]-1H-indole-2-carboxylate (14)

In a 50 mL two-necked round-bottomed flask equipped with a magnetic stirring bar, a rubber septum and an argon balloon was placed ethyl 6-bromo-3-hydroxy-1H-indole-2-carboxylate (125.4 mg, 0.44 mmol), DMAP (0.09 mmol, 10.8 mg), triethylamine (0.12 mL, 0.88 mmol) and CH2Cl2 (10 mL), and to it was added a solution of TBDMSCl (0.88 mmol, 132.6 mg) in CH2Cl2 (4 mL). After the mixture was stirred for 16 h at room temperature, it was concentrated in vacuo to give a crude oil, which was purified by silica gel column chromatography (hexane : ethyl acetate = 6 : 1) to give the title compound 14 (165.0 mg, 94%) as white crystals.

Yield 94% (165.0 mg); white crystals; mp 135–136 ºC; Rf = 0.54 (hexane : ethyl acetate = 4 : 1); 1H NMR (400 MHz, CDCl3) δ 0.21 (s, 6H), 1.09 (s, 9H), 1.43 (t, J = 6.9, 3H), 4.44 (q, J = 7.3, 2H), 7.19–7.17 (m, 1H), 7.47–7.49 (m, 2H), 8.65 (s, 1H); 13C NMR (100 MHz, CDCl3) δ –4.2, 14.7, 18.3, 25.7, 60.7, 114.4, 114.7, 120.0, 120.6, 121.5, 123.0, 133.8, 139.7, 161.7; IR (neat) 3313, 2952, 1675, 1568, 1472, 1316, 1240, 1145, 851, 783 cm⁻¹; HRMS (EI) calcd for C17H14BrNO3Si (M⁺) 397.0709 found 397.0707.

Ethyl 1-benzyl-6-bromo-3-[[tert-butyldimethylsilyloxy]-1H-indole-2-carboxylate (15)

In a 30 mL two-necked round-bottomed flask equipped with a magnetic stirring bar, a rubber septum and an argon balloon were placed methyl 2-amino-4-bromobenzoate (116.1, 120.7, 129.5, 132.7, 148.6, 167.9, 168.0; IR (neat) 3342, 2952, 1743, 1693, 1571, 1505, 1240, 1095, 1064, 769 cm⁻¹; HRMS (EI) calcd for C17H14BrNO3Si (M⁺) 397.0709 found 397.0707.

This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.
Ethyl 1-benzyl-6-bromo-2-methoxy-3-oxindoline-2-carboxylate (16)

In a 30 mL two-necked round-bottomed flask equipped with a magnetic stirring bar, a rubber septum and an argon balloon was placed NBS (59.1 mg, 0.33 mmol) and MeOH (6.0 mL), and to it was added a solution of ethyl 1-benzyl-6-bromo-3-[(tert-butyldimethylsilyl)oxy]-1H-indole-2-carboxylate (59.1 mg, 0.30 mmol) in MeOH (4 mL) at 0 °C. After the mixture was stirred for 15 min at 0 °C, to it was added saturated aqueous K2CO3 to quench the reaction. The whole mixture was extracted with ethyl acetate (50 mL x 3). The combined organic phases were washed with brine, dried over Na2SO4, and concentrated in vacuo to give a crude product, which was purified by silica gel column chromatography (n-hexane : ethyl acetate = 4 : 1) to give the title compound 16 (117.6 mg, 97%) as a yellow oil.

Yield 97% (117.6 mg); yellow oil; Rf = 0.42 (n-hexane : ethyl acetate = 4 : 1); 1H NMR (400 MHz, CDCl3) δ 1.09–1.13 (m, 3H), 3.27 (s, 3H), 3.96–4.10 (m, 2H), 4.47–4.60 (m, 2H), 6.90–7.05 (m, 2H), 7.29–7.37 (m, 5H), 7.44–7.48 (m, 1H); 13C NMR (100 MHz, CDCl3) δ 13.8, 46.6, 52.1, 121.7, 123.1, 126.0, 127.1, 127.7, 128.8, 134.2, 135.9, 161.4, 165.1, 193.5; IR (neat) 2930, 1704, 1606, 1472, 1418, 1367, 1257, 1119, 830, 781 cm⁻¹; HRMS (EI) calcd for C17H16BrNO3 (M)+ 403.0419 found 403.0414.

1-Benzyl-6-bromo-3-[(tert-butyldimethylsilyl)oxy]-1H-indol-2-yl methanol (18)

In a 30 mL two-necked round-bottomed flask equipped with a magnetic stirring bar, a rubber septum and an argon balloon was placed NBS (59.1 mg, 0.33 mmol) and MeOH (6.0 mL), and to it was added a solution of 1-benzyl-6-bromo-3-[(tert-butyldimethylsilyl)oxy]-1H-indole-2-carboxylate (23.0 mg, 0.05 mmol) in CH2Cl2 (5.0 mL), and to it was added dropwise DIBAL-H (0.09 mL, 0.30 mmol) in CH2Cl2 (5.0 mL) at 0 °C. After the mixture was stirred for 1 min at 0 °C, to it was added saturated aqueous K2CO3 to quench the reaction. The whole mixture was extracted with ethyl acetate (50 mL x 3). The combined organic phases were washed with brine, dried over Na2SO4, and concentrated in vacuo to give a crude product, which was purified by silica gel TLC (n-hexane : ethyl acetate = 3 : 1) to give the title compound 18 (19.6 mg, 88%) as a yellow green oil.

Yield 88% (19.6 mg); yellow green oil; Rf = 0.31 (n-hexane : ethyl acetate = 4 : 1); 1H NMR (400 MHz, CDCl3) δ 0.18 (s, 6H), 1.08 (s, 9H), 4.65 (d, J = 5.5 Hz, 2H), 5.38 (s, 2H), 6.92–6.94 (m, 2H), 7.14–7.42 (m, 6H); 13C NMR (100 MHz, CDCl3) δ –4.4, 18.2, 25.8, 46.8, 53.5, 112.4, 116.4, 119.8, 120.1, 122.2, 125.0, 125.7, 127.4, 128.8, 132.6, 135.3, 137.8; IR (neat) 3413, 2931, 2858, 1584, 1468, 1364, 1253, 1189, 1008, 829, 781 cm⁻¹; HRMS (EI) calcd for C28H38BrNO3Si (M)+ 445.1073 found 445.1073.

1-Benzyl-6-bromo-2-(hydroxymethyl)-2-methoxyindolin-3-one (17)

In a 30 mL two-necked round-bottomed flask equipped with a magnetic stirring bar, a rubber septum and an argon balloon was placed NBS (89.4 mg, 0.50 mmol) and MeOH (25.0 mL), and to it was added a solution of 1-benzyl-6-bromo-3-[(tert-butyldimethylsilyl)oxy]-1H-indol-2-yl methanol (203.8 mg, 0.46 mmol) in MeOH (4 mL) at 0 °C. After the mixture was stirred for 5 min at 0 °C, to it was added saturated aqueous K2CO3 to quench the reaction. The whole mixture was extracted with ethyl acetate (50 mL x 3). The combined organic phases were dried over Na2SO4, and concentrated in vacuo to give a crude product, which was purified on silica gel TLC (n-hexane : ethyl acetate = 3 : 1) to give the title compound 17 (150.4 mg, 90%) as a yellow green oil.

Yield 90% (150.4 mg); Rf = 0.19 (n-hexane : ethyl acetate = 4 : 1); 1H NMR (400 MHz, CDCl3) δ 3.12 (s, 3H), 3.65 (d, J = 0.0 Hz, 2H), 3.85–3.91 (m, 1H), 4.59 (s, 2H), 6.90–6.96 (m, 2H), 7.36–7.40 (m, 5H), 7.42–7.44 (m, 1H); 13C NMR (100 MHz, CDCl3) δ 45.2, 52.0, 63.9, 112.0, 114.5, 121.9, 122.7, 125.5, 126.7, 127.8, 129.4, 149.8, 213.3; IR (neat) 3462, 2929, 1716, 1606, 1472, 1312, 1092, 937, 755 cm⁻¹; HRMS (EI) calcd for C17H16BrNO3Si (M)+ 361.0314 found 361.0297.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research (B) and on Innovative Areas “Organic Synthesis Based on Reaction Integration. Development of New Methods and Creation of New Substances” from JSPS and MEXT.

Notes and references

1 [a] T. Kawasaki, A. Ogawa, Y. Takashima and M. Sakamoto, Tetrahedron Lett., 2003, 44, 1591; [b] P. N. Wyrembak and A. D. Hamilton, J. Am. Chem. Soc., 2009, 131, 4566; [c] K. Okuma, N. Matsunaga, N. Nagahora, K. Shioji and Y. Yokomori, Chem. Commun., 2010, 5822; [d] Y. Sun and R. Fan, Chem. Commun., 2010, 6834; [e] W. Sun, L. Hong and R. Wang, Chem.–Eur. J., 2011, 17, 6030; [f] A. Wetzell and F. Gagoss, Angew. Chem., Int. Ed., 2011, 50, 7354.

2 [a] I. Carletti, B. Banaigs and P. Amade, J. Nat. Prod., 2000, 63, 981; [b] S.-S. Wen, Z.-F. Zhou, J.-A. Xiao, J. Li, H. Xiang and H. Yang, New J. Chem., 2017, 41, 11503; [c] For a review see, G. W. Gribble in Progress in Heterocyclic Chemistry, ed by G. W. Gribble and J. A. Joule, Pergamon, Kidlington, vol. 15, 2003, pp. 58–74.

3 [a] P. S. Baran and E. J. Corey, J. Am. Chem. Soc., 2002, 124, 7904; [b] P.-L. Wu, Y.-L. Hsu and C.-W. Jao, J. Nat. Prod., 2006, 69, 1467; [c] L. A. Adams, M. W. N. Valente and R. M. Williams, Tetrahedron, 2006, 62, 5195; [d] D. D. O'Rell, F. G. H. Lee and V. Boekelheide, J. Am. Chem. Soc., 1972, 94, 3205; [e] S. Tsukamoto, H. Umaoka, K. Yoshikawa, T. Ikeda and H. Hirota, J. Nat. Prod., 2010, 73, 1438; [f] A. Karadeolian and M. A. Kerr, Angew. Chem., Int. Ed., 2010, 49, 1133; [g] A. Karadeolian and M. A. Kerr, J. Org. Chem., 2010, 75, 6830.
For the formation of hemiaminal ethers, see, (a) G. Li, F. R. Fronczek and J. C. Antilla, J. Am. Chem. Soc., 2008, 130, 12216; (b) K. Xu, Z. Wang, J. Zhang, L. Yu and J. Tan, Org. Lett., 2015, 17, 4476; (c) A. Beltran, E. Alvarez, M. M. Diaz-Requejo and P. J. Pereza, Adv. Synth. Catal., 2015, 357, 2821; (d) M. Li, B. Luo, Q. Liu, Y. Hu, A. Ganesan, P. Huang and S. Wen, Org. Lett., 2014, 16, 10; (e) H. Yu and J. Shen, Org. Lett., 2014, 16, 3204; (f) For a related work, see, T. Kano, T. Yurino, D. Asakawa and K. Maruoka, Angew. Chem., Int. Ed., 2013, 52, 5532.

For the bromination of silyl enol ethers, see, (a) R. H. Reuss and A. Hassner, J. Org. Chem., 1974, 39, 1785; (b) L. Blanco, P. Amicone and J. M. Conia, Synthesis, 1976, 194; (c) G. H. Fambly and T. H. Chan, Tetrahedron Lett., 1986, 27, 2563.

For the oxidative formation of iminium salts from amino ketene silyl acetics, see, S. Hata, H. Koyama and M. Shimizu, J. Org. Chem., 2011, 76, 9670.

For the reaction of 6-alkylation to α-imino esters in our laboratory, see, (a) P. H. B. França, D. P. Barbosa, D. L. da Silva, É. A. N. Ribeiro, A. E. G. Santana, B. V. O. Santos, J. M. Barbosa-Filho, J. S. S. Quintans, R. S. S. Barreto, L. J. Quintans-Junior and J. X. de Araújo-Júnior, BioMed Res. Int., 2014, 1.