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Synthesis of 4-substituted-3-Hydroxyquinolin-2(1H)-ones with anticancer activity

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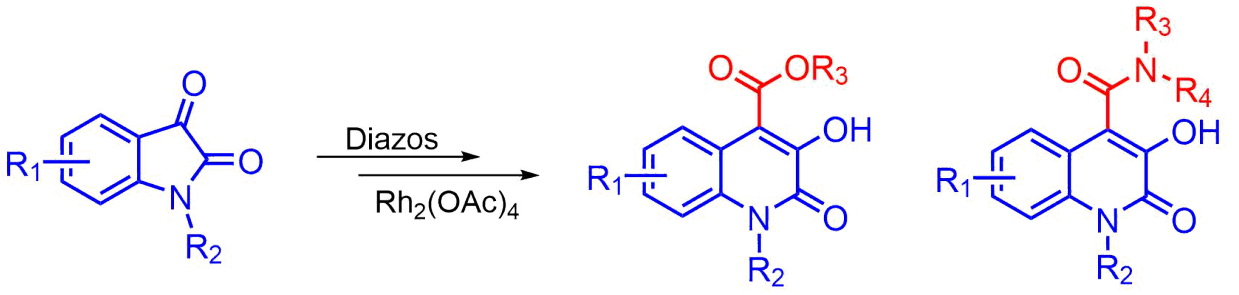
Eistert ring-expansion reaction | **Functionalized Anticancer 3HQs**



Yields up to 92%

IC_{50} up to 1.8 μM

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Synthesis of 4-Substituted-3-Hydroxyquinolin-2(1H)-ones with Anticancer Activity

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ABSTRACT

Herein we show that the 3-hydroxyquinolin-2(1*H*)-one (3HQ) core is a suitable platform to develop new compounds with anticancer activity against MCF-7 (IC_{50} up to 4.82 µM) and NCI-H460 (IC_{50} up to 1.8 µM) cancer cell lines. The ring-expansion reaction of isatins with diazo esters catalysed by di-rhodium(II) complexes proved to be a simple and effective strategy to synthesize 4-carboxylate-3HQs (yields up to 92%). 4-Carboxamide-3HQs were more efficiently prepared using NHS-diazoacetate in yields up to 88%. This innovative methodology enabled the construction of "peptidic-like" 3HQs, with several amino acid substituents. Among this series, the L-leucine derivative induced the cell death of MCF-7 (IC_{50} of 15.1 µM) and NCI-H460 (IC_{50} of 2.7 µM) cancer cell lines without causing any appreciable cytotoxicity against the non-cancer cell model (CHOK1).

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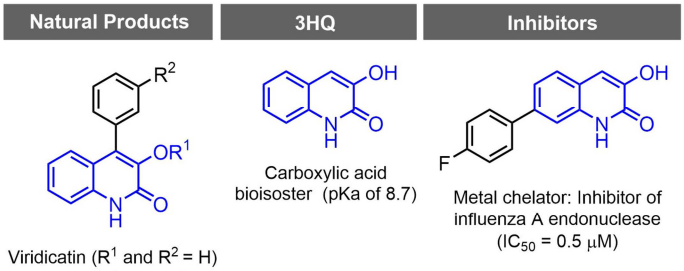
1. Introduction

The 3-hydroxyquinolin-2(1*H*)-one (3HQ) core is an important structural motif present in the natural products like viridicatin, viridicatol and 3-*O*-methyl viridicatin.[1–8] These metabolites, isolated from penicillium species, have been shown to inhibit the replication of human immunodeficiency virus and to be promising lead compounds for the development of new anti-inflammatory agents.[9,10] Furthermore, this unique heterocycle was recognized to be a valuable bioisoster for the carboxylic acid function of α-amino acids. This has triggered the interest towards the development of procedures for the synthesis of this class of compounds.[11] Although less acidic (pK_a of 8.7) than a carboxylic acid,[12,13] a series of 3HQs were prepared at Pfizer and shown to be potent inhibitors of the D-amino acid oxidase activity, eliciting similar binding interactions with the enzyme active site as the carboxylic acid containing inhibitors.[14] These discoveries were not left unnoticed, and recently this pharmacophore was found to bind to metal cofactors, by this way inhibiting the influenza A endonuclease.[15]

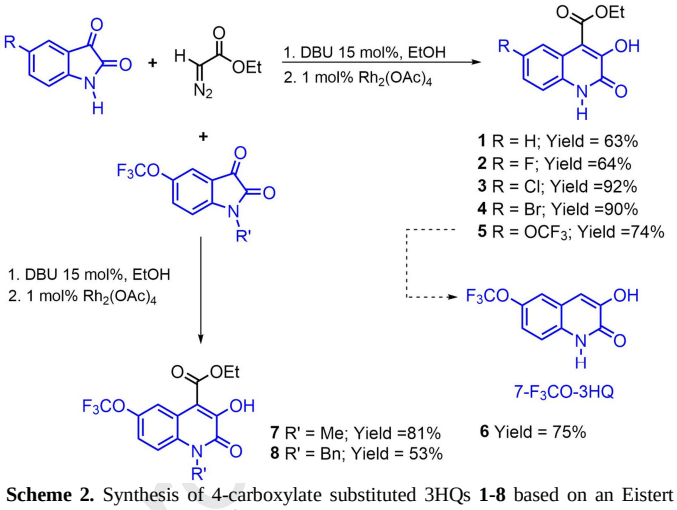
Rather surprisingly, and despite the many possibilities offered by the 3HQ core to develop new biologically active compounds, the use of this scaffold in the construction of anti-proliferative agents, remained basically unexplored. However, while developing new inhibitors of the HIV-1 reverse transcriptase associated RNase H activity, Bailly and co-workers observed that a series of 4-substituted 3HQs were significantly cytotoxic against non-cancer MT-4 cells, and this precluded their further use as antiviral agents.[16] Based on this observation, we envisioned that 4-carboxylate and 4-carboxamides substituted 3HQs could be further explored as a valuable platform to prepare innovative anticancer agents.



Scheme 1. The 3-hydroxyquinolin-2(1*H*)-one (3HQ) core present in the structure of naturally occurring compounds, as a carboxylic acid bioisoster and as an enzyme inhibitor.

2. Results and discussion

Aiming to test this hypothesis, we set out to prepare a small library of 4-substituted-3HQs to evaluate their potential anti-proliferative activity. As shown in Scheme 2, exploring our previously described Eistert ring-expansion reaction of isatins with diazo acetate (EDA) catalysed by $Rh_2(OAc)_4$,[17] 4-carboxylate-3HQs **1-5** were synthesised in good to excellent yields. Once prepared, this set of compounds was evaluated against a panel of breast cancer cells (MCF-7), human non-small lung cancer cells (NCI-H460) and human colorectal adenocarcinoma cells (HT-29) (Scheme 2, Table 1). This assay revealed that 3HQ **1-4** were non-active against the three cancer lines tested, though the 6- trifluoromethoxy -4-ethylacetate-3HQ **5** was could reduce the viability of the NCI-H460 cells by 8% at the concentration of 20 μM (Table 1). This result prompted us to explore the derivatization of the 3HQ scaffold bearing a trifluoromethoxy substituent on position 6. Structural modification of this core, by either decarboxylation or *N*-alkylation resulted in the loss of the observed antiproliferative activity, as indicated by the results of cell viability obtained in the presence of compounds **6**, **7** and **8**. Hence, we addressed the influence of the substituent at the position 4 on the activity of the heterocycle.



Scheme 2. Synthesis of 4-carboxylate substituted 3HQs **1-8** based on an Eistert ring-expansion reaction of isatins with diazo acetate (EDA) catalysed by $Rh_2(OAc)_4$.

Table 1. Anti-proliferative evaluation of compounds **1-8** against MCF-7, NCI-H460 and HT-29 cancer cell lines.

Compound	MCF-7	NCI-H460	HT-29
1	NA	NA	87%
2	NA	NA	NA
3	NA	NA	NA
4	86%	NA	NA
5	95%	52%	74%
6	NA	97%	NA
7	NA	NA	NA
8	NA	NA	NA

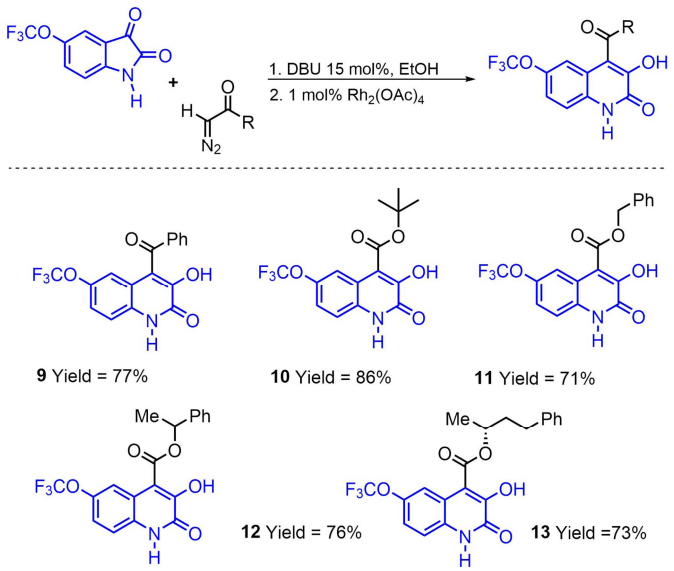
Percentage of cell-viability; NA – Non-active at the concentration of 20 μM

To study this, a series of diazo compounds were prepared and used in the Eistert ring-expansion reaction of the 5-trifluoromethoxy-isatin.[18] As shown in Scheme 3, this simple protocol afforded 3HQ **9-13** in yields ranging from 71 to 86%. Once prepared, the 3HQs were evaluated against the aforementioned panel of cancer cell lines. As shown in Table 2, the operated structural modification had a profound impact on the heterocycles activity. Promisingly, introducing a benzyl ester on compound **11** resulted in an increased activity against the three cancer cell lines with an IC_{50} as low as 1.8 μM, against NCI-H460 cells. Analogously, the 3HQ **13** featuring a slightly longer alkylic chain also showed an IC_{50} of 2.1 μM against the same cell line. However, the indiscriminate activity observed for these molecules, suggested the possibility of these 3HQs being also significantly toxic against non-cancer cell lines.

Table 2. Anti-proliferative evaluation of compounds **9-13** against MCF-7, NCI-H460 and HT-29 cancer cell lines.

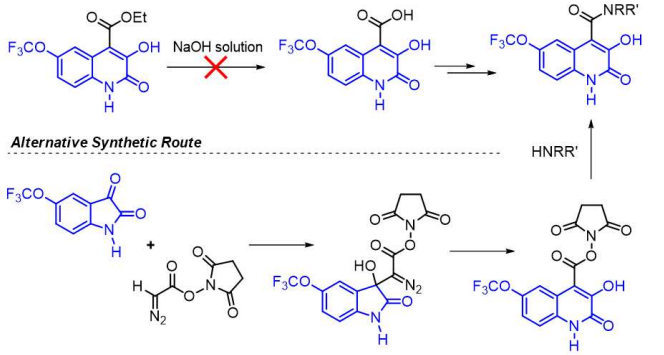
Compound	MCF-7	NCI-H460	HT-29
9	10.8±1.1	10.4±1.9	NA
10	13.4±2.5	6.0±1.0	NA
11	10.1±2.1	1.8±1.2	11.4±1.1
12	12.1±1.0	7.3±1.2	NA
13	16.0±1.2	2.1±1.1	NA

Determined IC_{50} of the compounds in MCF-7, NCIH460 and HT-29 cancer cell lines after 48 hours of incubation; NA – Non-active at the concentration of 20 µM



Scheme 3. Synthesis of 4-carboxylate substituted 3HQs **9**-**13** based on an Eistert ring-expansion reaction of isatins with diazo acetate (EDA) catalysed by di-rhodium complexes.

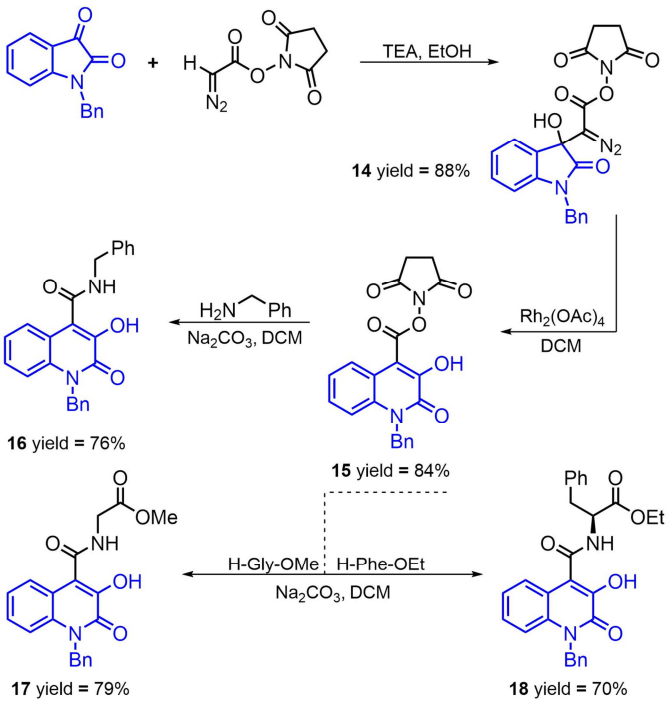
To study this, compound **12** was evaluated against the non-cancer Chinese hamster ovary cells (CHOK1) and, as expected, it proved to be quite cytotoxic to this model (IC_{50} of 5.6±1.0 µM). The incorporation of alkylic esters at position 4 of the scaffold clearly induced a higher anti-proliferative effect against cancer cells but regrettably, also a significant toxicity towards non-cancer cell lines. Therefore, to improve these compounds toxicity profile, we studied the anti-proliferative properties of 6-trifluoromethoxy-4-carboxamide-3HQs. This study was initiated with the synthesis of 4-carboxamides-3HQs following reported protocols, in which the ethyl ester is typically hydrolysed to the corresponding acid under basic conditions, and then coupled with primary and secondary amides *via* an acid chloride intermediate. Unfortunately, and despite our many attempts, when starting with 6-trifluoromethoxy-4-ethylacetate-3HQ **5**, this simple protocol invariably resulted in a decarboxylation process yielding compound **6** (Scheme 2).



Scheme 4. Possible synthetic routes to prepare 4-carboxamides-3HQs.

Interestingly, the reported synthesis of 4-carboxamides-3HQs starting from the corresponding carboxylic acid derivatives proceeds with poor yields (<40%), indicating the need to improve the synthetic methodologies to access these compounds.[16] Based on this, we conceived that a more direct route to prepare 4-carboxamide-3HQs would be to perform the Eistert ring-expansion reaction with NHS-diazo acetate,[19,20] followed by a simple amidation step (Scheme 4). We tested this idea adopting *N*-benzyl isatin as a model scaffold in order to avoid potential cross reactivity of the exposed amide group. To this end, *N*-benzyl isatin was reacted with NHS-diazo acetate under different basic conditions (see ESI). This step proved to be very reversible, though when using 20 mol% of TEA in EtOH, intermediate **14** precipitated from the reaction mixture, and was isolated in 88% yield without the need of chromatographic purification. Diazo intermediate **14** was then submitted to the ring expansion reaction catalysed by 0.5 mol% of $Rh_2(OAc)_4$. The reaction proceeded smoothly in DCM, and compound **15** was isolated by filtration in 84% yield. Finally, benzylamine was added to 3HQ **15** with sodium carbonate, and this afforded the targeted 4-carboxamide 3HQ **16** in a good yield. Notably, this method proved to be compatible with more complex amines, and glycine methyl ester and L-phenylalanine ethyl ester afforded the peptidic-like[12] 4-carboxamides-3HQs **17** and **18** in 79% and 70% yields respectively.

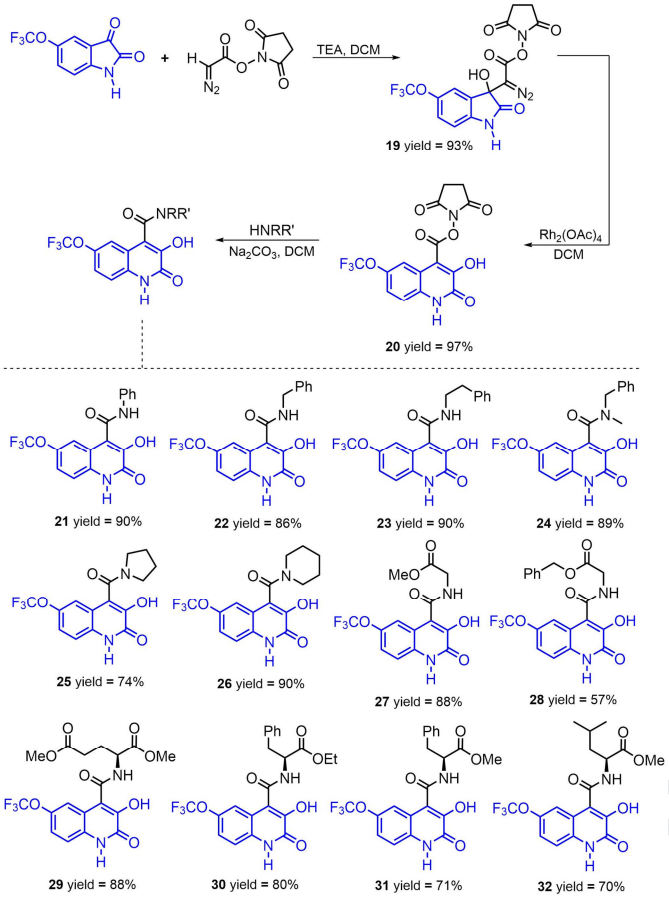
Once demonstrated the feasibility of this synthetic scheme, the same protocol was used to functionalize the 5-trifluoromethoxy -isatin (Scheme 3, Table 1). As shown in Scheme 5, the presence of an unprotected amide group was not detrimental for the preparation of the diazo intermediate **19** that was obtained in 93%, neither to the ring expansion step, that afforded the 3HQ heterocycle **20** almost quantitatively using 0.5 mol% of $Rh_2(OAc)_4$. As expected, primary and secondary amines also reacted smoothly with the 6-trifluoromethoxy -4-NHS-3HQ **20** to yield the carboxamides **21**-**26** in yields up to 90%. Similarly, protected amino acids also afforded the 4-carboxamides-3HQs **27**-**32** in good to excellent yields without any chromatographic step.



Scheme 5. Synthesis of 4-carboxamide-3HQs based on Eistert ring-expansion reaction of protected isatins with NHS-diazo acetate, followed by an amidation step.

Once established the preparation of 3HQ **21**-**32**, the anti-proliferative activity of these compounds was evaluated. Observation of the results shown in Table 3 indicates that 4-carboxamides-3HQs were shown to be less toxic against the non-cancer cell model (CHOK1) than the 4-carboxylate-3HQs series. For instance, benzylamide **22** was only slightly less potent

towards MCF-7 and NCI-H460 cancer cell lines than its benzyl ester counterpart **11**, while being clearly less toxic to CHOK1 cells at a concentration of 20 µM than compound **11** (Table 3).



Scheme 6. Synthesis of 4-carboxamide-3HQs based on Eistert ring-expansion reaction of 5- trifluoromethoxy -isatin with NHS-diazo acetate, followed by an amidation step.

This profile was even more pronounced in the case of amides **23** and **26** that showed a good selectivity towards the MCF-7 (IC_{50} of 4.8 µM) and NCI-H460 (IC_{50} of 7.3 µM) cancer cell lines respectively (Table 3), with negligible toxicity towards the CHOK1 cells. The "peptidic-like" 4-carboxamides-3HQs **30-32** were also active against the MCF-7 and NCI-H460 cell lines.

Table 3. Anti-proliferative evaluation of compounds **21-32** against MCF-7, NCI-H460 and CHOK1 cell lines.

COMPOUND	MCF-7	NCI-H460	CHOK1
21	NA	NA	NA
22	12.0±1.0	9.5±1.2	NA
23	4.8±1.2	NA	NA
24	17.5±2.4	NA	NA
25	NA	NA	ND
26	NA	7.3±1.2	NA
27	NA	NA	ND
28	12.6±1.1	NA	NA
29	NA	NA	ND
30	9.4±7.5	8.4±1.7	NA
31	9.5±1.0	11.4±1.1	NA
32	15.1±1.9	2.7±1.4	NA

Determined IC_{50} of the compounds in MCF-7 and NCIH460 cancer cell lines and CHOK1 non-cancer cell model after 48 hours of incubation; NA – Non-active at the concentration of 20 µM; ND – Not determined

In particular, compound **32** elicited an IC_{50} of 2.7 µM against the NCI-H460 cells (Table 3), which compares well with the best result obtained with the 4-carboxylate-3HQ series (Table 2). Consistently with what observed for the previously tested compounds, also this series of 3HQ derivatives did not elicit any anti-proliferative activity on HT-29 cancer cells. Due to their interesting activity both in NCI-H460 and MCF-7 cells, compound **13** and **32** were further tested for to their ability to induce cell death in these cell lines as measured by Lactate dehydrogenase (LDH) release, which is an indicator of plasma membrane damage. Interestingly, exposure to compound **13** or **32**, at IC_{50} and 2x IC_{50}, significantly increased general cell death in both cell lines, confirming the anticancer potential of these compounds (Figure 1, SI).

3. Conclusion

In this study, we address for the first time the cytotoxic potential of 3HQs. The Eistert ring-expansion reaction of isatins with diazo compounds catalysed by $Rh_2(OAc)_4$ was shown to be a versatile methodology to prepare 3HQs. The direct addition of structurally diverse diazo compounds to isatins enabled the construction of a series of 4-carboxylate-3HQs (in yields up to 86%) which were shown to present anti-proliferative activity against a panel of MCF-7, NCI-H460 and HT-29 cancer cell lines. Regrettably, this series of compounds also induced severe cytotoxicity against a model of non-cancer cell lines, which motivated us to evaluate the 4-carboxamide-3HQ counterparts. The troublesome preparation of these compounds was simplified by performing a ring expansion reaction of isatin derivatives with NHS-diazo acetate. This methodology afforded the targeted 4-carboxamides-3HQs in yields up to 90%, and this series of cytotoxic 3HQs were shown to have an improved selectivity towards MCF-7 (3HQ **23**, IC_{50} of 4.8 µM) and NCI-H460 (3HQ **26**, IC_{50} of 7.3 µM) cancer cell lines.

4. References and notes

[1] K.G. Cunningham, G.G. Freeman, , Biochem. J. 53 (1953) 328–332. [2] A. Bracken, A. Pocker, H. Raistrick, Biochem. J. 57 (1954) 587–595.

[3] J.H. Birkinshaw, M. Luckner, Y.S. Mohammed, K. Mothes, C.E. StickingsBiochem. J. 89 (1963) 196–202. [4] M. Luckner, K. Mothes, Tetrahedron Lett. 3 (1962) 1035–1039.

[5] M. Luckner, K. Mothes, No Title, Arch. Pharm. 18 (1963) 296.

[6] M.Y. Wei, R.Y. Yang, C.L. Shao, C.Y. Wang, D.S. Deng, Z.G. She, Y.C. Lin, 47 (2011) 322–325.

[7] D. Austin, M. Myers, J. Chem. Soc. 1 (1964) 1197–1198.

[8] I.Z. El Euch, M. Frese, N. Sewald, S. Smaoui, M. Shaaban, L. Mellouli, Med. Chem. Res. 27 (2018) 1085–1092. [9] N. Ribeiro, H. Tabaka, J. Peluso, L. Fetzer, C. Nebigil, S. Dumont, C.D. Muller, L. Désaubry, Bioorg. Med. Chem. Lett. 17 (2007) 5523–5524. [10] A. Heguy, P. Cai, P. Meyn, D. Houck, S. Russo, R. Michitsch, C. Pearce, B. Katz, G. Bringmann, D. Feineis, D.L. Taylor, A.S. Tyms, Antivir. Chem. Chemother. 9 (1998) 149–155.

[11] Y. Tangella, K. Manasa, N. hari krishna, B. Sridhar, A. Kamal, B. Nagendra Babu, Org. Lett. 20 (2018).

[12] C.B.M. Poulie, L. Bunch, ChemMedChem. 8 (2013) 205–215.

[13] C. Ballatore, D.M. Huryn, A.B. Smith, ChemMedChem. 8 (2013) 385–395.

[14] A.J. Duplantier, S.L. Becker, M.J. Bohanon, K.A. Borzilleri, B.A. Chrunyk, J.T. Downs, L.Y. Hu, A. El-

Kattan, L.C. James, S. Liu, J. Lu, N. Maklad, M.N. Mansour, S. Mente, M.A. Piotrowski, S.M. Sakya, S. Sheehan, S.J. Steyn, C.A. Strick, V.A. Williams, L. Zhang, J. Med. Chem. 52 (2009) 3576–3585.

[15] H.Y. Sagong, A. Parhi, J.D. Bauman, D. Patel, R.S.K. Vijayan, K. Das, E. Arnold, E.J. Lavoie, 2 (2013) 8–11.

[16] V. Suchaud, F. Bailly, C. Lion, E. Tramontano, F. Esposito, A. Corona, F. Christ, Z. Debyser, P. Cotelle, Bioorg. Med. Chem. Lett. 22 (2012) 3988–3992.

[17] R. Paterna, V. André, M.T. Duarte, L.F. Veiros, N.R. Candeias, P.M.P. Gois, European J. Org. Chem. (2013) 6280–6290.

[18] N.R. Candeias, R. Paterna, P.M.P. Gois, Chem. Rev. 116 (2016) 2937–2981.

[19] M.P. Doyle, A. V. Kalinin, J. Org. Chem. 61 (1996) 2179–2184.

[20] A. Ouihia, L. Rene, J. Guilhem, C. Pascard, B. Badet, J. Org. Chem. 58 (1993) 1641–1642.

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