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# Development of a synthetic route towards $N^4$,$N^9$-disubstituted 4,9-diaminoacridines: On the way to multi-stage antimalarials

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ABSTRACT

A multi-step synthetic route towards $N^4$,$N^9$-disubstituted 4,9-diaminoacridines that, to the best of our knowledge, has no precedence in the literature, has been developed. The target structures are likely to reveal interesting biological activities in the near future, not only due to their mepacrine-like core, but also because they embed simultaneously the pharmacophores of chloroquine and primaquine, antimalarial drugs that act at different stages of malaria infection.



The chemical synthesis of acridine-based compounds has attracted the attention and efforts of organic chemists ever since acridine (10-azaanthracene) itself was first isolated from coal tar in 1870, and soon after exploited as a scaffold for dyes used both in fabrics and as biological staining agents. Later, in 1912, in the so-called "dyes to drugs" journey, the antimicrobial properties of acridines was recognized, and since then a number of other potential therapeutic applications have been identified, including antiparasitic and antitumoral, among others [1,2]. In this connection, the first half of the XX$^{th}$ century emerged as a golden age for antimicrobial and antiparasitic acridines, respectively, due to the "antibiotic gap" prior to the establishment of penicillin as a first-line antibiotic in the late 1940s, and to the quinine shortage during World War II, which brought mepacrine (or quinacrine, **1**) into the antimalarial chemotherapy scenario [3,4] (Fig. 1).

Mepacrine's role as a first-line antimalarial was ephemeral, as it was soon superseded by chloroquine, a much safer, cheaper and effective antimalarial. Still, it has been rapidly repurposed for other conditions, finding applications as anti-helminthic [5], anti-rheumatic [6], anti-lupus [7], anti-cancer [8], anti-inflammatory [9], and female-sterilizing [10] agent. Its potential to treat Creutzfeld-Jakob disease was also investigated [11]. Interestingly, mepacrine has further gone through a "drug to dye" move, being explored as a fluorescent biolabeling agent [12], while the well-known acridine orange dye is presently being reconsidered as a valuable tool for rapid malaria diagnosis [13].

The wide-spectrum biological applications of acridines has fueled the development of new acridine-based structures and synthetic routes, as recently reported by others [14–17]. In the field of malaria chemotherapy, the widespread emergence of parasite strains resistant to chloroquine has revived the interest in mepacrine derivatives, all of which have the 9-amino-2-methoxy-6-chloroacridine core conserved but with different moieties linked to the 9-amino group [18–24]. Still, to the best of our knowledge, the synthesis of $N^4$,$N^9$-disubstituted 4,9-diaminoacridines (**2**) has not been reported. In view of this, and based on the assumption that compounds **2** may be potential multi-stage antimalarials, by incorporating the mepacrine core, and also by embedding both the chloroquine and the primaquine moieties (Fig. 2), we pursued their chemical synthesis.

A five-step synthesis starting from 4-chlorosalicylic acid (**3**) was first carried out (Scheme 1), to produce the 6,9-dichloro-2-methoxy-4-nitroacridine precursor of compounds **2**. Briefly, the carboxylic group in 4-chlorosalycilic acid **3** was protected upon

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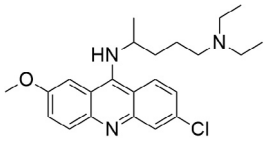

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**Fig. 1.** Structure of mepacrine, also known as quinacrine (**1**).

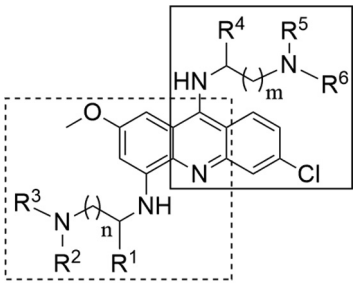

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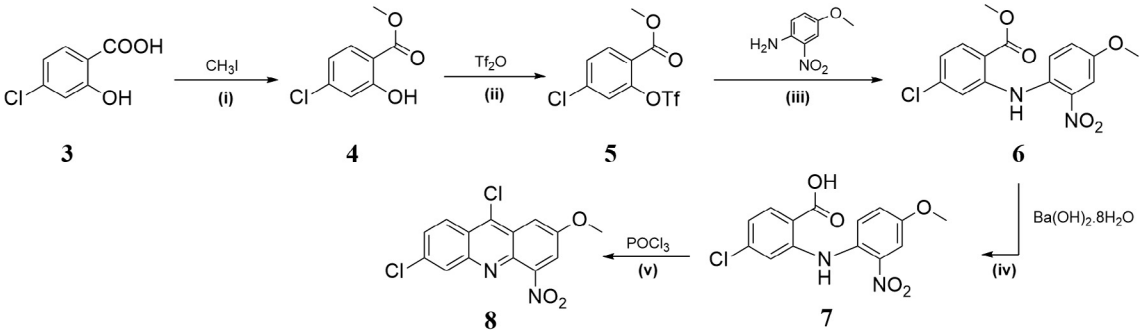
**Fig. 2.** General structure of the targeted $N^4$,$N^9$-disubstituted 4,9-diaminoacridines, **2**. Solid and dashed rectangles, respectively, delimit the structures of chloroquine ($R^4$ = Me, $R^5$ = $R^6$ = Et, m = 3) and primaquine ($R^1$ = Me, $R^2$ = $R^3$ = H, n = 3) antimalarials.

conversion into methyl ester **4**; this was accomplished by the reaction of **3** with excess iodomethane in the presence of $Cs_2CO_3$, according to Parrish and co-workers [25] (step i). Ester **4** was next reacted with trifluoroacetic (triflic) anhydride ($Tf_2O$) for activation of the phenol group *via* conversion into the corresponding triflate **5**, which was achieved in high yield (67%) following a procedure reported by Anderson and co-workers [20] (step ii). Triflate **5** was reacted with 4-methoxy-2-nitroaniline to afford intermediate **6** through a Buchwald-Hartwig amination, benefiting from the fact that this palladium-catalyzed cross-coupling between aryl triflates and substituted anilines is generally quite efficient [26]; to this end, the conditions described by Åhman and co-workers [27] were employed, and compound **6** was isolated in good (52%) yield (step iii). Then, the ester group in **6** was hydrolyzed with barium hydroxide octahydrate in methanol (MeOH), following a procedure by Anderson and co-workers [28] for quantitative formation, after acidification, of the corresponding carboxylic acid **7** (step iv). Finally, this acid was reacted with phosphoryl chloride for *in situ* generation of the acyl chloride intermediate that subsequently underwent an intramolecular Friedel-Crafts intramolecular acylation resulting in the formation of the desired 6,9-dichloro-2-methoxy-4-nitroacridine precursor (**8**) in good yield (70%, step v).

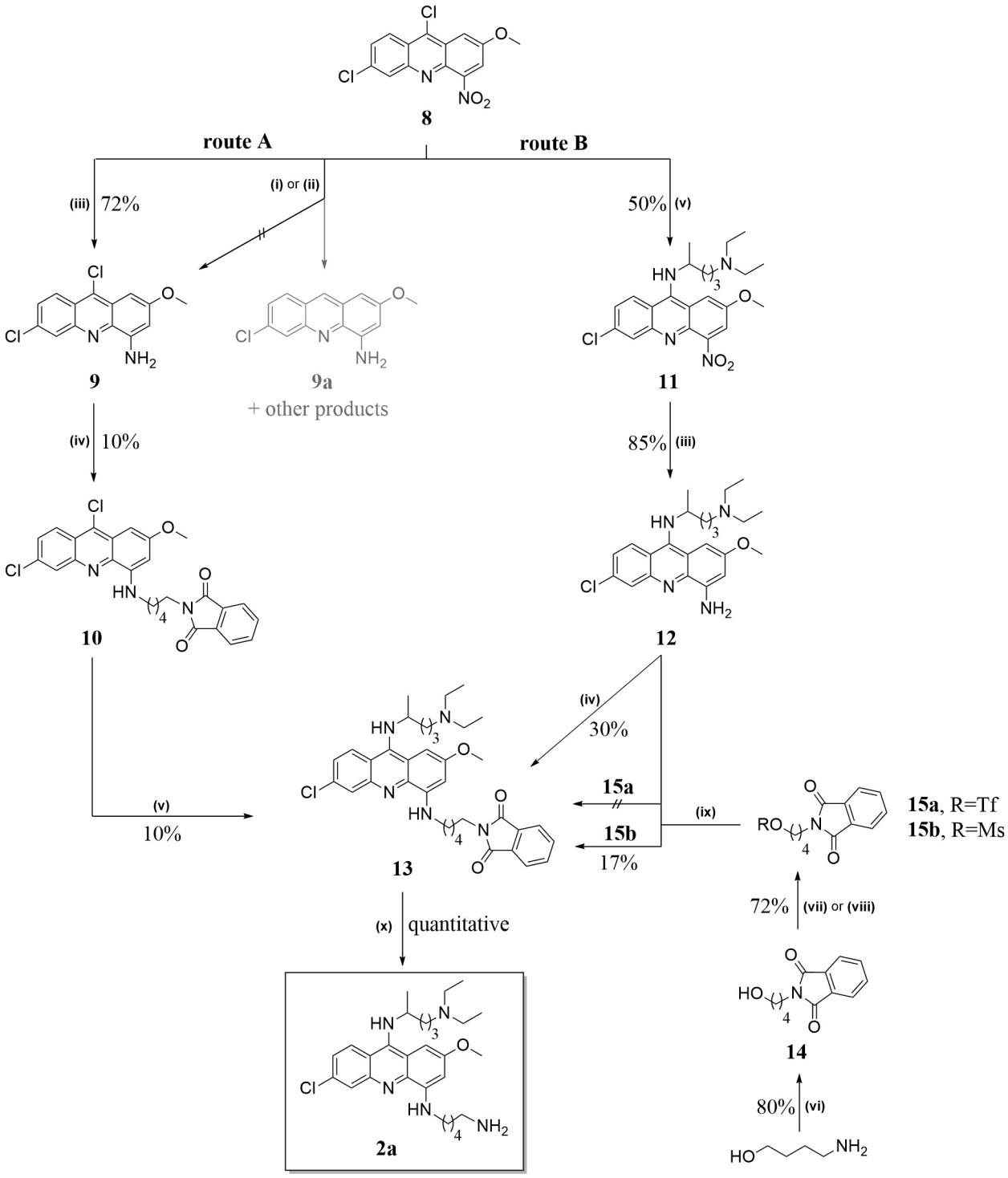
Starting from precursor **8**, two synthetic routes, see A and B in Scheme 2, towards $N^4$,$N^9$-disubstituted diaminoacridines **2** were developed, setting **2a** ($R^1$ = Me, $R^2$ = $R^3$ = $R^4$ = H, $R^5$ = $R^6$ = Et, m = n = 3 in **2**) as the target compound. The major difference between these routes was the order in which the $N^4$ and the $N^9$ aliphatic groups were introduced, i.e. in route A, introduction of the $N^4$-group preceded that of the $N^9$-group, and this was reversed in route B.

As shown in Scheme 2, the initial step in Route A was the reduction of the nitro group in **8** to the respective aniline **9**. The first two attempts made, namely, standard catalytic hydrogenation using $H_2$(g) and Pd/C [29–31] (step i), and transfer hydrogenation according to Mandal and co-workers [32], using triethylsilane (TES) as an *in situ* hydrogen source and Pd/C as catalyst (step ii), resulted in complex mixtures of products, from which it was possible to identify, by mass spectrometry (MS), the formation of a by-product of **9** corresponding to the loss of one chlorine atom (data not shown). Considering the expectedly higher reactivity of the C-9 position compared to the C-6 position in **9**, the by-product was likely 4-amino-6-chloro-2-methoxyacridine (**9a** in Scheme 2). Subsequent reduction of **8** using $SnCl_2$/HCl, according to a procedure by Scovill and co-workers, produced **9** in high yield (72%, step iii) [34]. Aniline **9** was next alkylated with *N*-(4-bromobutyl)phthalimide which, after optimization of the reaction conditions as summarized in Table 1, afforded the expected product **10**, using sodium acetate and ethanol (EtOH) as the base and solvent, respectively, and microwave heating at 120 °C for 2.5 h (Table 1, entry 7). However, the yield was quite low (10%, step iv); this was mostly due to the formation of multiple products, including 4-amino-6-chloro-2-methoxyacridin-9(10H)-one, detected by MS analysis (data not shown). In view of this, and considering that the high reactivity of the chlorinated C-9 position in the acridine moiety was likely to be the major factor underlying the complex behavior of the tested steps in route A, this route was abandoned and route B was pursued instead.

Route B started with the conversion of **8** into 4-nitromepacrine **11**, *via* an $S_NAr$ reaction using $N^1$,$N^1$-diethylpentan-1,4-diamine as the nucleophile (Scheme 2). After optimization of the reaction parameters such as reaction time, solvent, base, and temperature, **11** was produced in moderate yield (50%) through an adaptation of a procedure by Anderson and co-workers, based on the addition of excess phenol for intermediate formation of an aryl ether at C-9, which activates this position towards subsequent attack by the amine, and the use of $Cs_2CO_3$ as a base (step v). The nitro group in **11** was next reduced to aniline **12** in high yield (85%), using the $SnCl_2$/HCl method employed in Route A (step iii). Alkylation



**Scheme 1.** Synthesis of 6,9-dichloro-2-methoxy-4-nitroacridine **8**, precursor to the target compound **2a**. Reagents and conditions: (i) $CH_3I$ (5 equiv.), $Cs_2CO_3$ (0.5 equiv.), DMF, rt, 90 min; (ii) $Tf_2O$ (1.5 equiv.), TEA (2 equiv.), $CH_2Cl_2$, $N_2$ atmosphere, −25 °C, 30 min; (iii) 4-methoxy-2-nitroaniline (1.2 equiv.), $Cs_2CO_3$ (1.4 equiv.), $Pd(OAc)_2$ (0.05 equiv.), *rac*-BINAP (1.8 equiv.), toluene, $N_2$ atmosphere, 120 °C, 5 h; (iv) (1) $Ba(OH)_2$·$8H_2O$ (1.5 equiv.), MeOH, 90 °C, 2 h; (2) 1 M aq. HCl; (v) $POCl_3$ (34 equiv.), 120 °C, 2.5 h.




**Scheme 2.** Synthetic routes towards target compound **2a**. (i) $H_2$ (50 psi), Pd/C, MeOH, rt, 5 h; (ii) TES, Pd/C, MeOH, rt, 30 min; (iii) $SnCl_2$ (5 equiv.), 37% aq. HCl, 0 → 40 °C, 30 min; (iv) *N*-(4-bromobutyl)phthalimide (3 equiv.), $CH_3COONa$ (3 equiv.), EtOH, MW heating (100 W, 120 °C) in a pressurized vial (100 psi), 2.5 h; (v) phenol (15 equiv.), $Cs_2CO_3$ (1 equiv.), anhydrous DMSO, 4 Å molecular sieves, 100 °C, 2 h, then $N^1$,$N^1$-diethylpentan-1,4-diamine (4 equiv.), 100 °C, 4 h; (vi) phthalic anhydride (1 equiv.), dioxane, 100 °C, overnight; (vii) $Tf_2O$ (1.5 equiv.), TEA, (2 equiv.), $CH_2Cl_2$, $N_2$ atmosphere, −25 °C, 30 min; (viii) TEA (2 equiv.), MsCl (1.3 equiv.), EtOAc, 0 °C → rt, 10 min; (ix) pre-activation with $K_2CO_3$ (1 equiv.) in anhydrous MeCN, −25 °C, $N_2$ atmosphere, 1 h, then addition to **12**, 120 °C, overnight; (x) hydrazine monohydrate (40 equiv.), THF, 80 °C, 72 h.

of aniline **12** with *N*-(4-bromobutyl)phthalimide was then carried out using the conditions optimized in Route A (step iv), which produced **13** in 30% yield, a 3-fold higher alkylation yield than *via* Route A. Still, the use of other *N*-butylphthalimide derivatives to achieve this alkylation was further investigated. To this end, 4-aminobutan-1-ol was reacted with phthalic anhydride to produce **14** (step vi), which was reacted with either $Tf_2O$ or MsCl to afford the respective triflate **15a** (step vii) or mesylate **15b** (step viii), but only the latter was successfully obtained. Aniline **12** was then reacted with **15b**, which afforded the desired product **13** in moderate yield (17%, step ix). The difficulty in obtaining compound **15a** was unsurprising, as primary alkyl triflates are prone to undergo

**Table 1**
Optimization of the reaction conditions for $N^4$-alkylation using *N*-(4-bromobutyl)phthalimide (Scheme 2, step iv).

| Entry | Solvent | Reaction time (h) | Temperature (°C) | MW power (W) | Pressure (psi) | Yield **10** (%) |
|---|---|---|---|---|---|---|
| 1 | EtOH | 120 | 80 | – | ~14.7[a] | 9.3 |
| 2 | ACN | 120 | 80 | – | ~14.7[a] | – |
| 3 | EtOH | 168 | 80 | – | ~14.7[a] | 10 |
| 4 | EtOH | 2 | 100 | 100 | 100[b] | – |
| 5 | EtOH | 4 | 120 | 100 | 100[b] | 7.4 |
| 6 | EtOH | 3 | 120 | 100 | 100[b] | 8.2 |
| 7 | EtOH | 2.5 | 120 | 100 | 100[b] | 10 |

[a] Atmospheric pressure, *ca.* 1 atm ~ 14.7 psi.
[b] Pressurized and sealed reaction vials.

elimination reactions. One possible way to circumvent this limitation would be to run the reaction in THF and use 2,6-di-*tert*-butyl-4-methyl pyridine rather than triethylamine (TEA) as the base [35]. Usually, under these conditions, the conjugate acid salt precipitates from the reaction medium, which would minimize elimination issues that might occur with TEA. However, because the alkylation yield using the corresponding mesylate (step ix) was only about half the yield of the alkylation using *N*-(4-bromobutyl)phthalimide (step iv), we did not pursue this alternative pathway. Finally, compound **13** was submitted to hydrazinolysis using excess hydrazine monohydrate in THF at reflux; this delivered the target final compound **2a** in excellent yield (100%, step x).

$N^4$,$N^9$-disubstituted 4,9-diaminoacridines **2** are likely to possess multi-stage antimalarial activity, considering both (i) their mepacrine-like core structure that further embeds features of chloroquine, which acts on blood-stage parasites, and of primaquine, which targets parasite liver-stage forms and gametocytes (*c.f.*, Fig. 2), and (ii) reports from our group [36] and others [33], on antimalarial acridines displaying *in vitro* dual-stage activity and *in vivo* liver-stage activity on Rhesus monkeys, respectively. Confirmation of this hypothesis is being pursued in ongoing studies, and the *in vitro* activity of compound **2a** against blood, liver, and gametocyte stages of *Plasmodium* parasite development is presently under investigation. Preliminary *in vitro* data (undisclosed) indicate that compound **2a** retains the blood- and liver-stage activity of chloroquine and primaquine, respectively. Synthetic routes towards $N^4$, $N^9$-disubstituted 4,9-diaminoacridines **2** are unprecedented, and may pave the way towards novel bioactive compounds of therapeutic interest. The synthesis of a much larger set of compounds **2**, i.e. analogues of **2a**, is under investigation for subsequent *in vitro* identification of multi-stage antimalarial hits, and the establishment of relevant structure-activity relationships. Findings thereof will be communicated, and the synthetic route herein reported will be revised to increase yield and scale.

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## Appendix A. Supplementary data

Supplementary data (synthetic procedures, and structural data on compounds synthesized) to this article can be found online at https://doi.org/10.1016/j.tetlet.2019.03.052.

## References

[1] M. Gensicka-Kowalewska, G. Cholewiński, K. Dzierzbicka, RSC Adv. 7 (26) (2017) 15776–15804.
[2] X. Li, B. Li, C. Gao, Y. Jiang, Expert Opin. Ther. Pat. 24 (6) (2014) 647–664.
[3] R.J. Joy, Med. Hist. 43 (2) (1999) 192–207.
[4] C. Teixeira, N. Vale, B. Pérez, A. Gomes, J.R.B. Gomes, P. Gomes, Chem. Rev. 114 (22) (2014) 11164–11220.
[5] G. Panic, U. Duthaler, B. Speich, J. Keiser, Int. J. Parasitol. Drugs Drug Resist. 4 (3) (2014) 185–200.
[6] R.I. Rynes, Br. J. Rheumatol. 36 (7) (1997) 799–805.
[7] S. Benoit, M. Goebeler, Acta Derm-Venereol. 95 (5) (2015) 596–599.
[8] R. Ehsanian, C. Van Waes, S.M. Feller, Cell Commun. Signal. 9 (1) (2011) 13.
[9] A.A. Chumanevich, E.E. Witalison, A. Chaparala, A. Chumanevich, P. Nagarkatti, M. Nagarkatti, L.J. Hofseth, Oncotarget 7 (33) (2016) 52928–52939.
[10] J. Lippes, Contraception 92 (2) (2015) 91–95.
[11] M.D. Geschwind, A.L. Kuo, K.S. Wong, A. Haman, G. Devereux, B.J. Raudabaugh, D.Y. Johnson, C.C. Torres-Chae, R. Finley, P. Garcia, J.N. Thai, H.Q. Cheng, J.M. Neuhaus, S.A. Forner, J.L. Duncan, K.L. Possin, S.J. Dearmond, S.B. Prusiner, B.L. Miller, Neurology 81 (23) (2013) 2015–2023.
[12] H. Cai, F. Mullier, B. Frotscher, M.E. Briquel, M. Toussaint, F. Massin, T. Lecompte, V. Latger-Cannard, Semin. Thromb. Hemost. 42 (3) (2016) 282–291.
[13] M. Kimura, I. Teramoto, C.W. Chan, Z.M. Idris, J. Kongere, W. Kagaya, F. Kawamoto, R. Asada, R. Isozumi, A. Kaneko, Malaria J. 17 (1) (2018) 72.
[14] C. Felip-Leon, O. Martinez-Arroyo, S. Diaz-Oltra, J.F. Miravet, N. Apostolova, F. Galindo, Bioorg. Med. Chem. Lett. 28 (5) (2018) 869–874.
[15] H. Wu, Z. Zhang, N. Ma, Q. Liu, T. Liu, G. Zhang, J. Org. Chem. 83 (20) (2018) 12880–12886.
[16] O. Sharhan, T. Heidelberg, N.M. Hashim, A.A. Salman, H.M. Ali, S.N. Jayash, RSC Adv. 8 (68) (2018) 38995–39004.
[17] K.E. Berger, G.M. McCormick, J.A. Jaye, C.M. Rozeske, E.H. Fort, Molecules 23 (11) (2018) 2867.
[18] K. Chibale, H. Haupt, H. Kendrick, V. Yardley, A. Saravanamuthu, A.H. Fairlamb, S.L. Croft, Bioorg. Med. Chem. Lett. 11 (19) (2001) 2655–2657.
[19] A. Sparatore, N. Basilico, S. Parapini, S. Romeo, F. Novelli, F. Sparatore, D. Taramelli, Bioorg. Med. Chem. 13 (18) (2005) 5338–5345.
[20] M.O. Anderson, J. Sherrill, P.B. Madrid, A.P. Liou, J.L. Weisman, J.L. DeRisi, R.K. Guy, Bioorg. Med. Chem. 14 (2) (2006) 334–343.
[21] L. Guetzoyan, F. Ramiandrasoa, H. Dorizon, C. Desprez, A. Bridoux, C. Rogier, B. Pradines, M. Perree-Fauvet, Bioorg. Med. Chem. 15 (9) (2007) 3278–3289.
[22] L. Guetzoyan, X.M. Yu, F. Ramiandrasoa, S. Pethe, C. Rogier, B. Pradines, T. Cresteil, M. Perree-Fauvet, J.P. Mahy, Bioorg. Med. Chem. 17 (23) (2009) 8032–8039.
[23] X.M. Yu, F. Ramiandrasoa, L. Guetzoyan, B. Pradines, E. Quintino, D. Gadelle, P. Forterre, T. Cresteil, J.P. Mahy, S. Pethe, ChemMedChem 7 (4) (2012) 587–605.
[24] B. Pérez, C. Teixeira, A.S. Gomes, I.S. Albuquerque, J. Gut, P.J. Rosenthal, M. Prudêncio, P. Gomes, Bioorg. Med. Chem. Lett. 23 (3) (2013) 610–613.
[25] J.P. Parrish, E.E. Dueno, S.-I. Kim, K.W. Jung, Synthetic Commun. 30 (15) (2000) 2687–2700.
[26] Z. Wang, Comprehensive Organic Name Reactions and Reagents, Wiley, 2009.
[27] J. Åhman, S.L. Buchwald, Tetrahedron Lett. 38 (36) (1997) 6363–6366.
[28] M.O. Anderson, J. Moser, J. Sherrill, R.K. Guy, Synlett 2004 (13) (2004) 2391–2393.
[29] D. Wang, D. Astruc, Chem. Rev. 115 (13) (2015) 6621–6686.
[30] F. Nerozzi, Heterogeneous Catalytic Hydrogenation vol. 6 (2012) 236–241.
[31] L. ČervenÝ, Chem. Eng. Commun. 83 (1) (1989) 31–63.
[32] P.K. Mandal, J.S. McMurray, J. Org. Chem. 72 (17) (2007) 6599–6601.
[33] J.P. Scovill, D.L. Klayman, T.S. Woods, T.R. Sweeney, J. Med. Chem. 22 (10) (1979) 1164–1167.
[34] P.A.S. Smith, J. Polym. Sci. 7 (2) (A1 1969,) 795–796.
[35] S.A. Ross, M. Pitié, B. Meunier, J. Chem. Soc. Perkin Trans. 1 (4) (2000) 571–574.
[36] A. Gomes, B. Pérez, I. Albuquerque, M. Machado, M. Prudêncio, F. Nogueira, C. Teixeira, P. Gomes, ChemMedChem 9 (2) (2014) 305–310.