Tetrahedron Letters xxx (xxxx) xxx

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Application of PEG_{400} in the one-pot synthesis of 7-[4-alkyl- or (hetero)aryl-1*H*-1,2,3-triazol-1-yl]thieno[3,2-*b*]pyridines *via* S_NAr and Cu(I)-Catalyzed Azide-Alkyne Cycloaddition and preliminary evaluation of their anti-tumour activity

Juliana M. Rodrigues [a], Ricardo C. Calhelha [b], Isabel C.F.R. Ferreira [b], Maria-João R.P. Queiroz [a,*]

[a] *Centro de Química-Universidade do Minho (CQUM), Escola de Ciências, Campus de Gualtar, 4710-057 Braga, Portugal*
[b] *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal*

ARTICLE INFO

Article history:
Received 4 March 2020
Revised 30 March 2020
Accepted 1 April 2020
Available online xxxx

Keywords:
PEG_{400}
Thieno[3, 2-b] pyridines
CuAAC
1, 4-Disubstituted-1,2, 3-triazoles

ABSTRACT

Several novel 7-[4-alkyl- or (hetero)aryl-1*H*-1,2,3-triazol-1-yl]thieno[3,2-*b*]pyridines were prepared in good to high yields, using the environmentally friendly solvent PEG_{400} in a one-pot procedure from 7-chlorothieno[3,2-*b*]pyridine to form the corresponding azide *via* S_NAr with NaN_3, followed by Cu(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC) using different types of alkynes. This one-pot reaction in PEG_{400} starting from a halogenated heteroaromatic system is reported for the first time and demonstrated a wide scope of application for alkynes. Preliminary anti-tumour activity on human tumour cell lines using the prepared 1,4-di(hetero)aryl-1,2,3-triazoles was evaluated, together with their toxicity in non-tumour cells. Among the tested compounds the most promising one was a 2-ethynylpyridine derivative.

Introduction

The thieno[3,2-*b*]pyridine and 1,2,3-triazole motifs are important cores and many heterocycles with incorporated triazoles have been shown to possess a wide range of biological activities [1a-c].

Since 2010 our research group has reported different Pd and/or Cu catalyzed syntheses of thieno[3,2-*b*]pyridine functionalized derivatives either on the pyridine ring *via* C—C [2,3], C—N [4], and C—O [5a,b] couplings or on the thiophene ring *via* C—N coupling [6], Cu(I) Azide-Alkyne Cycloaddition (CuAAC) [7], and Sonogashira coupling followed by 6-*endo-dig* cyclization to give the corresponding tricyclic pyranones [8]. Evaluation of the anti-tumour activity of the prepared compounds was performed using several human tumour cell lines and for the most promising compounds insights into their mechanisms of action were achieved (e.g. cell cycle alterations and apoptosis).

The synthesis of *o*-, *m*- and *p*-aminothioethers *via* the S_NAr reaction of 7-chlorothieno[3,2-*b*]pyridine (**1**) has also been described by our group. They reacted with substituted arylisocyanates to give the corresponding 1,3-diarylurea derivatives, and *m*-aminothioether thieno[3,2-*b*]pyridine-1,3-diarylureas gave the best results as antiangiogenic (Tyr kinase inhibitors of VEGFR-2) [9a] and anti-tumour compounds [9b], depending on the arylisocyanate substitution.

For some years, alternative reaction media have been used to replace volatile organic solvents and polyethylene glycols (PEGs) have emerged as a green reaction media for several types of reactions due to their low cost, thermal stability and biodegradability [10a,b].

Since 2006 there have been various reports in the literature using one-pot procedures for the synthesis of 1,4-disubstituted 1,2,3-triazoles in PEG_{400} *via* azide intermediates obtained from different substrates such as Baylis-Hillman acetates [11a], epoxides or *N*-tosylaziridines [11b], and α-tosyloxy ketones [11c], using NaN_3, a terminal alkyne and CuI.

In 2010, Kumar and co-workers reported the regioselective synthesis of 1,4-di(hetero)aryl-1*H*-1,2,3-triazoles in a one-pot sequence from di(hetero)aryliodonium triflate salts in PEG_{400}, *via* the corresponding azides followed by CuAAC [11d]. To the best of our knowledge there are no further reports of the use of PEG_{400} in this type of reaction from heteroaromatic substrates.

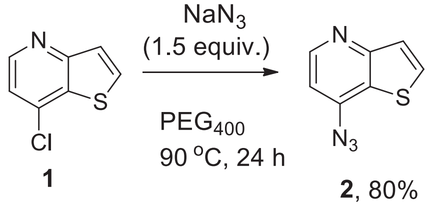
Herein, we describe the one-pot synthesis of novel 7-[4-alkyl- or (hetero)aryl]-1*H*-1,2,3-triazol-1-yl]thieno[3,2-*b*]pyridines in

---

* Corresponding author.
E-mail address: mjrpq@quimica.uminho.pt (M.-J.R.P. Queiroz).

https://doi.org/10.1016/j.tetlet.2020.151900
0040-4039/© 2020 Elsevier Ltd. All rights reserved.

Please cite this article as: J. M. Rodrigues, R. C. Calhelha, I. C. F. R. Ferreira et al., Application of PEG_{400} in the one-pot synthesis of 7-[4-alkyl- or (hetero)aryl-1*H*-1,2,3-triazol-1-yl]thieno[3,2-*b*]pyridines *via* S_NAr and Cu(I)-Catalyzed Azide-Alkyne Cycloaddition and preliminary evaluation of their anti-tumour activity, Tetrahedron Letters, https://doi.org/10.1016/j.tetlet.2020.151900



Scheme 1. Synthesis of azide **2** from compound **1** in PEG$_{400}$ using the optimal conditions (Table 1, entry 3).

good to high yields, from 7-chlorothieno[3,2-*b*]pyridine (**1**) and NaN$_3$ to give the corresponding azide, followed by CuAAC [12a,b] with different type of alkynes in PEG$_{400}$.

Results and discussion

First, commercially available compound **1** was converted into the corresponding novel azide **2** *via* S$_N$Ar with NaN$_3$ in PEG$_{400}$ after optimization of the reaction conditions (Scheme 1, Table 1 entries 1–3).

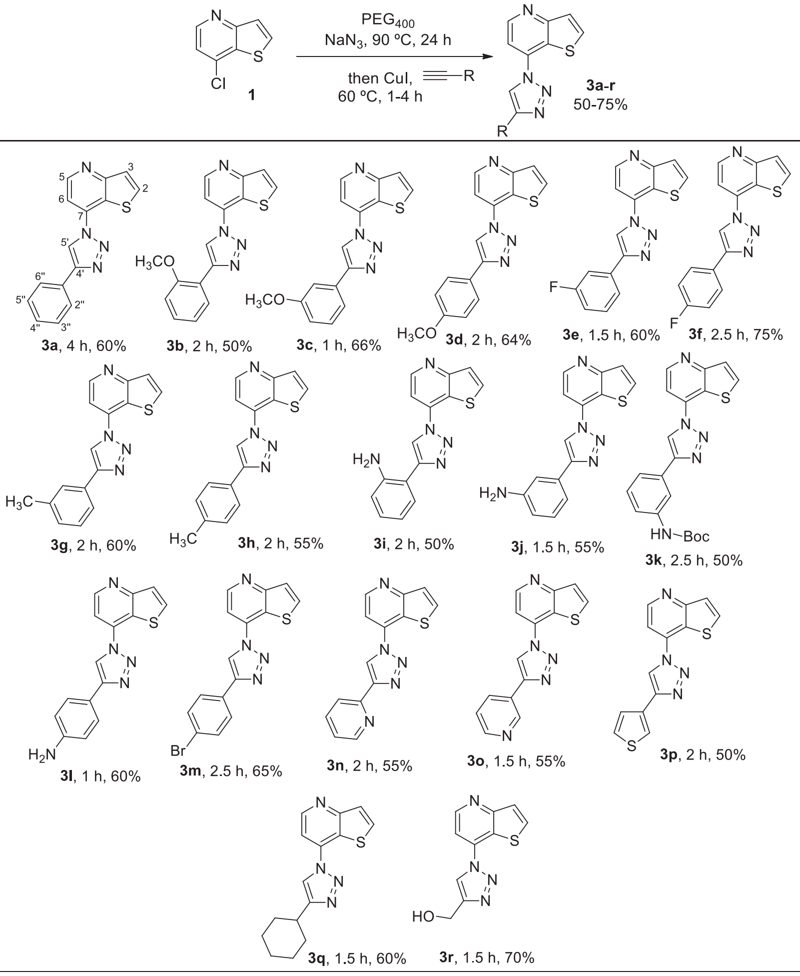
It is noteworthy that the reaction did not occur in acetonitrile either at rt for 24 h or at 60 °C for 48 h (Table 1, entries 4 and 5).

The optimal conditions for the synthesis of azide **2** were then applied to the first step of the one-pot reaction. The CuAAC step attempts at rt or at 40 °C were not very successful but at 60 °C compounds **3** formed rapidly (1–4 h, following the reaction by TLC). The novel compounds **3a-r** were obtained in good to high yields in a two-step, one-pot procedure from compound **1**, after column chromatography (Table 2) [13]. In the reaction work-up,

Table 1
Optimization of the reaction conditions for the synthesis of azide **2**.

Entry	Solvent	Temperature (°C)	Time (h)	NaN$_3$ (equiv.)	Yield (%)
1	PEG$_{400}$	rt	24	1.5	30
2	PEG$_{400}$	60	24	1.5	48
3	PEG$_{400}$	90	24	1.5	80
4	MeCN	rt	24	1.5	0
5	MeCN	60	48	1.5	0

Table 2
One-pot synthesis of compounds **3a-r** from compound **1** and alkynes (1 equiv.) using CuI (20 mol%) [13].



Please cite this article as: J. M. Rodrigues, R. C. Calhelha, I. C. F. R. Ferreira et al., Application of PEG$_{400}$ in the one-pot synthesis of 7-[4-alkyl- or (hetero)aryl-1*H*-1,2,3-triazol-1-yl]thieno[3,2-*b*]pyridines *via* S$_N$Ar and Cu(I)-Catalyzed Azide-Alkyne Cycloaddition and preliminary evaluation of their anti-tumour activity, Tetrahedron Letters, https://doi.org/10.1016/j.tetlet.2020.151900

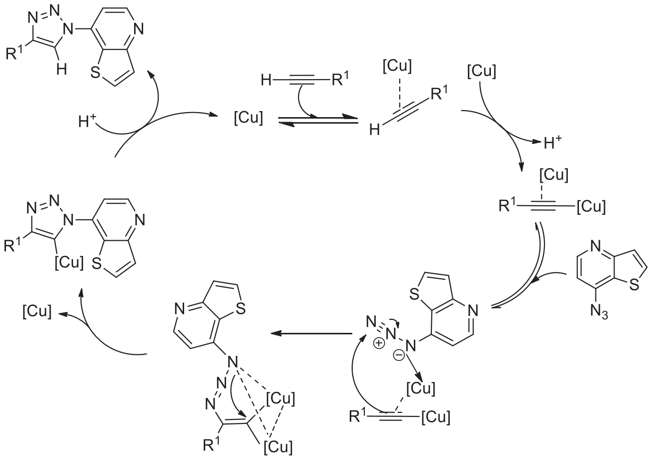


Fig. 1. Catalytic cycle proposed by Fokin and co-workers [16], adapted to our system.

washing the organic phase (AcOEt) with a saturated sodium thiosulphate solution [14] enhanced PEG$_{400}$ removal thus increasing the compound yield.

The mechanism of the CuAAC reaction has been studied recently and dinuclear copper intermediates were characterized by ESI(+)-MS/MS.[15] These experiments were in agreement with the studies performed earlier by Fokin and co-workers in 2013 using calorimetry and metal isotope crossover methods that allowed the deduction of the involvement of unstable, non-isolable dinuclear copper intermediates, instead of mononuclear ones, in a new proposed catalytic cycle to regioselectively give 1,4-disubstituted-1*H*-1,2,3-triazoles (Fig. 1, adapted to our system).[16]

Considering Fig. 1, it is difficult to find a relationship between the structure of the alkyne and the corresponding yield. In the first part of the CuAAC mechanism an alkyne that easily forms the Cu acetylide is important (favoured by EWGs), but for linkage with the azide a high electronic density in the triple bond is relevant (favoured by EDGs). Among the aryl derivatives (Table 2) and compared to **3a**, the compounds obtained in the lowest yields were those with EDGs in the *ortho*- position **3b** (*o*-OCH_3) and **3i** (*o*-NH_2), which may be due to steric hindrance, and in the *meta*-position **3j** (*m*-NH_2) and **3k** (*m*-NHBoc), which does not increase the electronic density in the triple bond. The similar yields of the latter compounds indicate that there is no advantage to prepare **3k** as a precursor of **3j**.

There are no significant differences in the yields for compounds substituted at the *meta*- and *para*- positions with OCH_3 (**3c** and **3d**) and CH_3 (**3g** and **3h**), respectively, both being obtained in very good yields. Nonetheless, substitutions with F and NH_2 gave better yields for the *para*-substituted compounds **3f** (*p*-F) and **3l** (*p*-NH_2) than for the corresponding *meta*-substituted compounds **3e** and **3j**. The compound with bromine in the *para*-position **3m** was also obtained in very good yield (65%). The results for the halogenated compounds seem to be in agreement with their negative inductive effect that favours the formation of the copper acetylide and with the positive mesomeric effect of their lone pairs in the *para*-position that induces an increase in the electronic density at the triple bond. The latter effect is important for the EDG NH_2 in **3l** (60%).

The yields of the heteroaromatic alkyne derivatives (**3n-p**) were similar despite the different electronic character of the pyridine and thiophene rings.

Alkyl derivatives **3q** and **3r** were also prepared from cyclohexylethynyl and propargylic alcohol in good to high yields, respectively, possibly due to their positive inductive effects.

The 1,4-di(hetero)aryl-1,2,3-triazoles **3a-p** were screened for their anti-tumour potential in four human tumour cell lines: HeLa (cervical carcinoma), MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung carcinoma), HepG2 (hepatocellular carcinoma) and in non-tumour cells: PLP2 (Porcine Liver Primary cell line), using the Sulforhodamine B colorimetric assay [2–6]. The GI_{50} concentrations, which inhibited 50% of the cell growth, were determined and although the results were not encouraging for most compounds (see ESI for all examples), **3b** (*o*-OCH_3) and **3n** (pyridin-2-yl) showed the lowest GI_{50} values in all tumour cell lines tested without toxicity in PLP2 at their GI_{50} values. The anti-tumour ellipticine used as the standard is also cytotoxic for PLP2 showing a low GI_{50} value in this non-tumour cell line (Table 3). Compound **3n** was the most promising, particularly in the HepG2 cell line (GI_{50} = 7.51 μM).

Conclusion

We have prepared novel 7-[4-alkyl- and (hetero)aryl-1*H*-1,2,3-triazol-1-yl]thieno[3,2-*b*]pyridines **3a-r** in good to high yields using the environmentally friendly solvent PEG$_{400}$ in a one-pot procedure from the chlorinated heteroaromatic compound **1** *via* the corresponding azide **2** obtained by S_NAr with NaN_3, followed by CuAAC using different alkynes. This one-pot reaction constitutes the first example starting from a halogenated heteroaromatic system and demonstrated a wide scope of application for different type of alkynes.

The anti-tumour potential of compounds **3a-p** was evaluated and compound **3n** showed the lowest GI_{50} values ($\leq$15 μM) in all the human tumour cell lines tested without toxicity at those concentrations in the non-tumour cell line PLP2. The search for new anti-tumour compounds without toxicity continues to be very important in order to avoid the secondary effects of chemotherapy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Fundação para a Ciência e Tecnologia (FCT)–Portugal financially supports CQUM (UID/QUI/686/2019), CIMO-IPBragança (UID/AGR/690/2019), the research project PTDC/QUI-QFI/28020/2017 (POCI-01-0145-FEDER-028020) also financed by European

Table 3
GI_{50} values of the most promising anti-tumour compounds **3b** and **3n**.

GI_{50} (μM)[a]					
	HeLa	**MCF-7**	**NCI-H460**	**HepG2**	**PLP2**
3b	13.49 ± 0.44	23.15 ± 0.97	15.81 ± 0.39	15.06 ± 1.13	113.92 ± 7.86
3*n*	12.99 ± 0.58	15.13 ± 0.59	12.60 ± 0.8	7.51 ± 0.48	91.94 ± 5.23
Ellipticine	4.75 ± 0.55	3.69 ± 0.16	7.96 ± 0.25	8.1 ± 0.54	8.57 ± 0.13

[a] GI_{50} concentrations correspond to the mean ± SD of at least three independent experiments. Ellipticine was used as a positive control.

Please cite this article as: J. M. Rodrigues, R. C. Calhelha, I. C. F. R. Ferreira et al., Application of PEG$_{400}$ in the one-pot synthesis of 7-[4-alkyl- or (hetero)aryl-1*H*-1,2,3-triazol-1-yl]thieno[3,2-*b*]pyridines *via* S_NAr and Cu(I)-Catalyzed Azide-Alkyne Cycloaddition and preliminary evaluation of their anti-tumour activity, Tetrahedron Letters, https://doi.org/10.1016/j.tetlet.2020.151900

Regional Development Fund (ERDF), COMPETE2020 and Portugal2020, the PTNMR network also supported by Portugal2020 and the PhD grant of J.M.R. (SFRH/BD/115844/2016) also financed by ESF (European Social Fund) and HCOP (Human Capital Operational Programme).

Appendix A. Supplementary data

Experimental procedures, characterization of compounds **2**, **3a-r** including ^{1}H, ^{13}C, ^{19}F NMR spectra, and preliminary anti-tumour evaluation. Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2020.151900.

References

[1] a) S.G. Agalave, S.R. Maujan, V.S. Pore, Chem. Asian J. 6 (2011) 2696–2718;
b) A. Lauria, R. Delisi, F. Mingoia, A. Terenzi, A. Martorana, G. Barone, A. Maria, Eur. J. Org. Chem. (2014) 3289–3306;
c) D. Dheer, V. Singh, R. Shankar, Bioorg. Chem. 71 (2017) 30–54.

[2] M.-J.R.P. Queiroz, R.C. Calhelha, L.A. Vale-Silva, E. Pinto, R.T. Lima, M.H. Vasconcelos, *Eur. J. Med. Chem.* 45 (2010) 5628–5634.

[3] M.-J.R.P. Queiroz, R.C. Calhelha, L.A. Vale-Silva, E. Pinto, G.M. Almeida, M.H. Vasconcelos, Eur. J. Med. Chem. 46 (2011) 236–240.

[4] M.-J.R.P. Queiroz, R.C. Calhelha, L.A. Vale-Silva, E. Pinto, M.S. Nascimento, Eur. J. Med. Chem. 45 (2010) 5732–5738.

[5] a) M.-J.R.P. Queiroz, S. Dias, D. Peixoto, A.R.O. Rodrigues, A.D.S. Oliveira, P.J.G. Coutinho, L.A. Vale-Silva, E. Pinto, E.M.S. Castanheira, J. Photochem. Photobiol. A: Chem. 238 (2012) 71–80;
b) M.-J.R.P. Queiroz, D. Peixoto, R.C. Calhelha, P. Soares, T. dos Santos, R.T. Lima, J.F. Campos, R.M.V. Abreu, I.C.F.R. Ferreira, M.H. Vasconcelos, Eur. J. Med. Chem. 69 (2013) 855–862.

[6] R.C. Calhelha, I.C.F.R. Ferreira, D. Peixoto, R.M.V. Abreu, L.A. Vale-Silva, E. Pinto, R.T. Lima, M.I. Alvelos, M.H. Vasconcelos, M.-J.R.P. Queiroz, Molecules 17 (2012) 3834–3843.

[7] J.M. Rodrigues, M.-J.R.P. Queiroz, Synthesis. 38 (2016) 2904–2910.

[8] J.M. Rodrigues, P. Buisson, J.M. Pereira, I.M. Pinheiro, T. Fernández-Marcelo, M. H. Vasconcelos, S. Berteina-Raboin, M.-J.R.P. Queiroz, Tetrahedron 75 (10) (2019) 1387–1397.

[9] a) V.A. Machado, D. Peixoto, R. Costa, H.J.C. Froufe, R.C. Calhelha, R.M.V. Abreu, I.C.F.R. Ferreira, R. Soares, M.-J.R.P. Queiroz, Bioorg. Med. Chem. 23 (2015) 6497–6509;
b) V.A. Machado, D. Peixoto, M.-J.R.P. Queiroz, R. Soares, J. Cell. Biochem. 117 (2016) 2791–2799.

[10] a) J. Chen, S.K. Spear, J.G. Huddleston, R.D. Rogers, Green Chem. 7 (2005) 64–82, Reviews on the use of PEG as a green reaction media, see:;
b) E. Colacino, J. Martinez, F. Lamaty, L.S. Patrikeeva, L.L. Khemchyan, V.P. Ananikov, I.P. Beletskaya, Coord. Chem. Rev. 256 (2012) 2893–2920.

[11] a) B. Sreedhar, P.S. Reddy, N.S. Kumar, Tetrahedron Lett. 47 (2006) 3055–3058;
b) G. Kumaraswamy, K. Ankamma, A. Pitchaiah, J. Org. Chem. 72 (2007) 9822–9825;
c) D. Kumar, V.B. Reddy, R.S. Varma, Tetrahedron Lett. 50 (2009) 2065–2068;
d) D. Kumar, V.B. Reddy, Synthesis 10 (2010) 1687–1691.

[12] V.V. Rostovtsev, L.G. Green, V.V. Fokin, K.B.A. Sharpless, Angew. Chem. Int. Ed. 41 (2002) 2596–2599;
b) C.W. Tornøe, C. Christensen, M. Meldal, J. Org. Chem. 67 (2002) 3057.

[13] General procedure for the one-pot synthesis of 7-(4-substituted-1*H*-1,2,3-triazol-1-yl)thieno[3,2-*b*]pyridines (**3a-r**): In a round bottom flask with PEG$_{400}$ (2 mL), compound **1** (100 mg, 5.90 mmol) and NaN$_3$ (1.5 equiv.) were added and the mixture was stirred at 90 ^{o}C for 24 h (controlled by TLC). Then, CuI (20 mol%), and the alkyne (1 equiv.) were added and the mixture was stirred at 60 ^{o}C for 1-4 h (controlled by TLC). After cooling the mixture was poured onto ice, ethyl acetate (5 mL) was added and the phases were separated. The aqueous phase was extracted with ethyl acetate (5 mL) and the combined organic phases were washed with a saturated sodium thiosulphate solution (2 × 10 mL). The organic phase was dried (MgSO$_4$), filtered and the removal of solvent gave oils. These were submitted to dry flash or column chromatography to isolate the corresponding product (Table 2).

[14] X. Wang, H. Gong, Z. Quan, L. Li, H. Ye, Synth. Comm. 41 (2011) 3251–3258.

[15] C. Iacobuci, S. Reale, J.-F. Gal, F. De Angelis, Angew. Chem. Int. Ed. 54 (2015) 3065–3068.

[16] B. Worrel, J.A. Malik, V.V. Fokin, Science 340 (2013) 457–460.

Please cite this article as: J. M. Rodrigues, R. C. Calhelha, I. C. F. R. Ferreira et al., Application of PEG$_{400}$ in the one-pot synthesis of 7-[4-alkyl- or (hetero)aryl-1*H*-1,2,3-triazol-1-yl]thieno[3,2-*b*]pyridines *via* S$_N$Ar and Cu(I)-Catalyzed Azide-Alkyne Cycloaddition and preliminary evaluation of their anti-tumour activity, Tetrahedron Letters, https://doi.org/10.1016/j.tetlet.2020.151900