RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2021, **11**, 2320

Solid-phase reactive chromatography (SPRC): a sustainable method for the synthesis of benzimidazol-diphenyl-2-imino-thiazolidine-4-ols (hemiaminals) which are active against lung cancer†

V. M. Bangade, *[ab] P. R. Mali[a] and H. M. Meshram *[a]

The concept of a green approach has been applied in the reaction, separation and solidification of a single unit. The products, 2-benzimidazol-diphenyl-2-imino-thiazolidine-4-ols, are purely hemiaminals. They have a tendency to decompose at a high rate. The solid-phase reactive chromatographic technique has been applied to avoid extensive liquid–liquid extraction and stabilised the product by precipitation in solvents. Benzimidazole phenyl thiourea and phenacyl bromide have been used as starting materials in this study. Both the starting materials are adsorbed on dry silica and packed in a glass column with a systematic arrangement of layers. A directly solid product was precipitated in cold hexane. Anticancer activities have been recorded against four cell lines, human colon, prostate, lung and breast cancer, with reference to doxorubicin as a standard. These compounds show a promising effect on human lung cancer, products **B9** (IC_{50} = 3.890 μM) and **B10** (IC_{50} = 2.798 μM) and **B13** (IC_{50} = 3.140 μM) which very close to doxorubicine (IC_{50} = 1.750 μM). It was observed that fluoro phenyl functionalities are effective compared to trifluoro methyl phenyl functionalities for anticancer activities. These small molecules lose their activities, except fluorination, on bulky substitution. This convenient metal-free approach is highly efficient for unstable hemiaminals such as the potent anticancer benzimidazol-diphenyl-2-imino-thiazolidine-4-ols.

Received 24th October 2020
Accepted 29th November 2020

DOI: 10.1039/d0ra09082d

rsc.li/rsc-advances

Introduction

The solid-supported on-column reactions are extremely popular because of their interesting applications like separation and purification by skipping liquid–liquid extraction in a single unit. Industrially viable chemical reactions have been developed in a manner that used recyclable solvents and reagents. Because of bulky batches, industrial techniques avoid the extraction process. Synthetic chemists are attentive towards cumulative single unit reactions for directly converting reactants into a pure product. There are important reactions performed in a column using solid phase reagents and catalysts:[1] asymmetric synthesis of beta-lactam,[2] Wittig and Horner–Emmons reaction,[3] esterification,[4] metathesis,[5] Suzuki coupling reaction,[6] and organocatalyzed Diels–Alder reaction.[7] The continuous flow method is one of the sequential column methods in which reagents and catalysts on a solid phase support are loaded onto sequential columns.[8]

Compounds with a tetrahedral carbon attached to both amino nitrogen and hydroxyl groups are assigned as hemiaminals (carbinolamines).[9] These are highly unstable intermediates in the reaction of amine with carbonyl compounds. In the synthesis of thiazoles, hemiaminals appeared only in the mechanism point of view but were difficult to isolate as stable compounds.[10,11] Synthesis of a stable hemiaminal is a hefty task for a synthetic chemist.

A literature survey reveals that 2-imino-thiazolidine-4-ols can be prepared by the reaction of thiourea and 2-bromo-acetophenone.[12] Recently, Dalmal *et al.* synthesized 2-imino-4-(trifluoromethyl)thiazolidin-4-alcohols (hemiaminals) using 3-bromo-1,1,1-trifluoropropan-2-one, followed by subsequent modifications for the various derivatives of isoxazoles, propargylamine and triazoles, which are biologically important compounds.[13] Not only Gunal *et al.* isolated hemiaminals[10] but also Tuncel and Dogan synthesized chiral hemiaminals by the reduction of 2-iminothiazolidine-4-ones using $LiAlH_4$.[9] Hemiaminals can be stabilized using synthetic receptors,[14] dendrimers[15] and triazole rings.[16]

[a]*Medicinal Chemistry and Pharmacology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad, 500007, India*

[b]*Department of Chemistry, The Institute of Science, Dr Homi Bhabha State University, 15, Madame Cama Road, Mumbai-32, India. E-mail: vikasmb2009@gmail.com*

† Electronic supplementary information (ESI) available: Characterization data, of compound, anticancer activities and single crystal X-ray analysis data. CCDC 1041069. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d0ra09082d

In this study, we stabilized benzimidazol-diphenyl-2-imino-thiazolidine-4-ols (hemiaminals) as the main products using benzimidazol-phenyl thiourea derivative 1a and substituted 2-bromo-acetophenone. Benzimidazole is a very important heterocyclic component in medicinal chemistry and synthetic chemistry. It is a unique bicyclic member of heterocyclic compound for its nucleophilic and electrophilic reactions. The reaction proceedings are diverse compared to other heterocyclic compounds. In the reported list of the WHO (in year 2019), triclabendazole is a medically approved essential medicine used for fascioliasis and paragonimiasis in treating liver flukes.[17] Tetra-substituted benzimidazole derivatives have anti-urease activity.[18] Benzimidazole derivatives are used for multipurpose activities, such as antiviral, antitumor, antipyretic, and anti-ulcer activities. Recently, Ozdemir *et al.* studied a series of benzimidazole-piperazine hybrids for cytotoxic activity against human lung cancer and breast cancer cell lines.[19] Thus, the synthesis of benzimidazole-based medicinally important compounds is a robust target for a chemist.

It was observed that the reported methods for the synthesis of 2-imino-thiazolidine-4-ol are acceptable but there are many parameters that should be under modification for the best results of hemiaminals. The time required for the reaction, separation, purification and stability of product are major factors responsible for the quality work of 2-imino-thiazolidine-4-ol. The reported methods are not applicable to unstable hemiaminals such as benzimidazol-diphenyl-2-imino-thiazolidine-4-ol, which is a strong anticancer agents. Thus, it is appreciated to develop a more viable and easy method for synthesizing 2-imino-thiazolidine-4-ol. By compiling these major factors and the combined advantage of benzimidazole and 2-imino-thiazolidine, we envisaged to synthesize benzimidazole-diphenyl-2-imino-thiazolidine-4-ol by column chromatography. To our knowledge, there is no report for the synthesis of hemiaminals such as benzimidazole-diphenyl-2-imino-thiazolidine-4-ol by reaction, separation and solidification in a single unit.

There are different structural hemiaminals synthesized but benzimidazole-diphenyl-2-imino-thiazolidine-4-ol hemiaminal (**B1**) is exclusively different than other hemiaminals shown in Fig. 1.

Fig. 1 shows the hemiaminals of trifluoromethyl 2-bromoacetophenone, **Ac** and **Ad**, which are highly stable. Hemiaminal **Ad** may be highly stable due to benzimidazole ring. Similarly, hemiaminal **B1** is more stable than **Aa** because it has benzimidazole ring and **Aa** will appear as a mechanism point of view. Though **B1** is stable in a solid state, it has an unstable nature in the solution form. Both **Ac** and **Ad** are highly stable in solid and solution. However, because of the benzimidazole ring, it may be possible that **Ad** is stable than **Ac**.

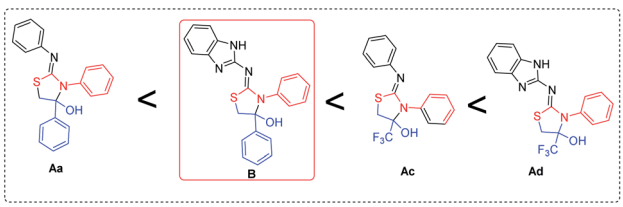
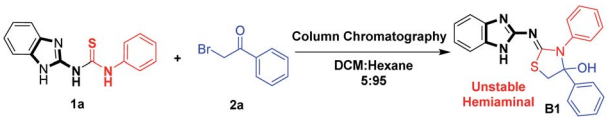


Fig. 1 Stability order of hemiaminals with respect to **B1**.



Scheme 1 Synthesis of benzimidazol-diphenyl-2-imino-thiazolidine-4-ol (hemiaminal) by column chromatography.

We disclose herein an industrially viable method for the synthesis of benzimidazole-diphenyl-2-imino-thiazolidine-4-ol (hemiaminal) by regular column chromatography, which combines reaction, separation and stabilization of product by solidification in a cumulative single unit (Scheme 1). Note that amino thiazolidine-4-ols show promising anticancer activities.

Results and discussion

We Initiated our efforts by attempting the reaction of 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-phenylthiourea **1a** and 2-bromoacetophenone **2a** in THF at room temperature, and within a minute we verified the progress of the reaction. The reaction was monitored by TLC; to our surprise, it was completed with a high rate of reaction. The reaction was extracted by ethyl acetate and separated by column chromatography. At the time of separation by column chromatography, we identified that the original product started to decompose. Literature survey and our practical approaches reveal that 2-imino-thiazolidine-4-ol usually undergoes decomposition.

As the rate of reaction was very high, due to the problem of decomposition, we planned a silica-adsorbed column-based reaction. Benzimidazol-phenyl thiourea **1a** was dissolved in minimum THF and adsorbed on silica (Merck 60–120 mesh silica gel). 2-Bromoacetophenone **2a** was then dissolved in minimum THF and adsorbed on a silica gel. Both adsorbed silica of **1a** and **2a** were packed in a glass column, which was eluted by 5% (DCM: hexane). The eluent was then collected in

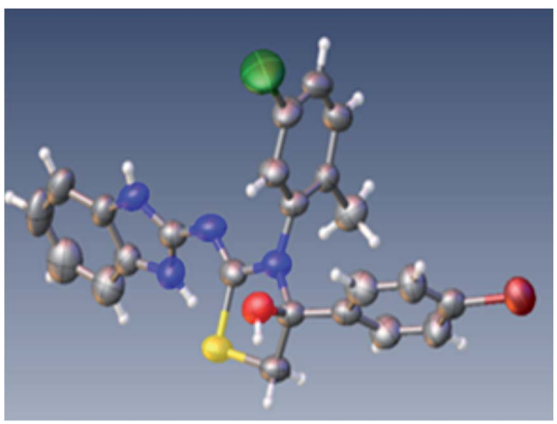


Fig. 2 Single crystal structure of **B12**.

ice-cold hexane. After some time, we reported that a milky precipitate started to appear in ice cold hexane. The column was eluted till the precipitate appeared. The solid precipitate was then filtered out with a sintered funnel, and the filtrate was evaporated under the vacuum partially such that only DCM was evaporated and the remaining product precipitated in hexane. The product was then filtered and dried using a vacuum pump. The product was characterized by NMR, ESI-mass, HRMS and finally confirmed as benzimidazole-2-imino-thiazolidine-4-ol (Fig. 2) by single crystal X-ray diffraction study.[21]

The column was prepared in such a manner that the total length of the glass column was 100 cm, and there were five different silica layers we have packed in the column. After a cotton plug at the bottom close to the stopcock, the first layer was packed by dry silica up to 5 cm for good separation. Then, the second layer was packed by adsorbed silica of thiourea **1a** (should be long layer, up to 30–40 cm long). The third layer was packed by dry silica gel up to 1–2 cm long. The fourth layer was packed with silica adsorbed by **2a** up to 05 cm long and was covered by 1–2 cm long dry silica followed by a cotton plug to stabilize the silica layer (Fig. 3).

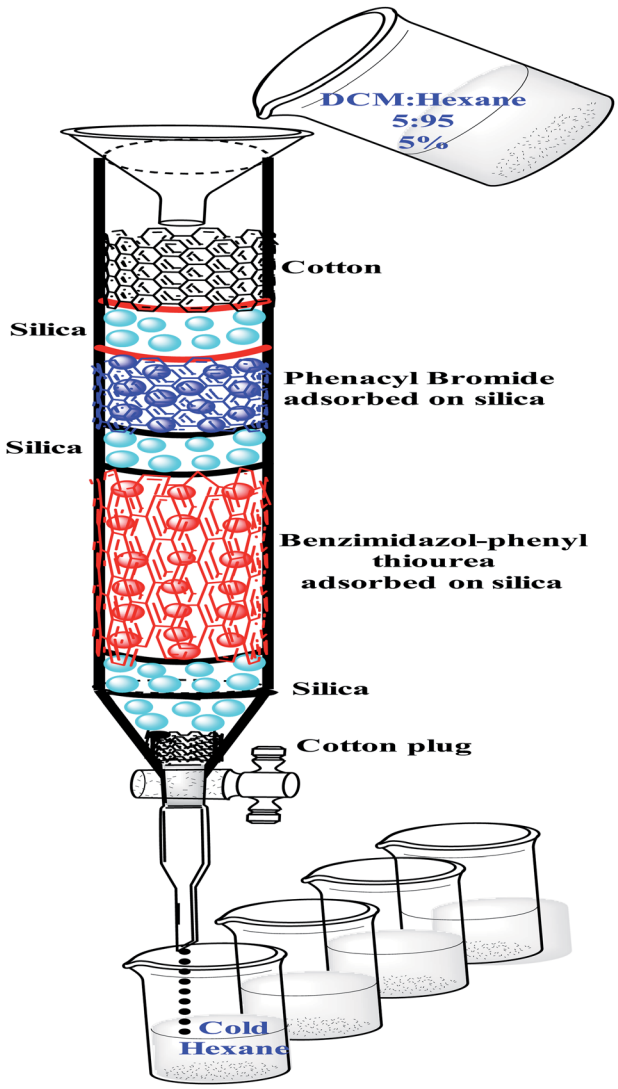


Fig. 3 Schematic diagram of column chromatography for the synthesis of benzimidazol-diphenyl-2-imino-thiazolidine-4-ol.

The reaction was screened for a combination of solvents in column chromatography. First, we attempted with 5% ethyl acetate : hexane, but we could not get a good yield because the product could not properly precipitate and was difficult to recover the stable product from solvent during vacuum evaporation at a low temperature. 5% DCM : hexane was best for good product yield because the product easily precipitated in cold hexane and DCM, and then easily evaporated at a low temperature followed by precipitation. The 5% was justified from TLC of the reaction such that the reaction in the column was completed in the stipulated time. We have screened the percentage of solvent from 1% to 10% DCM: hexane, and finally confirmed that 5% was suitable for optimum conversion of the product.

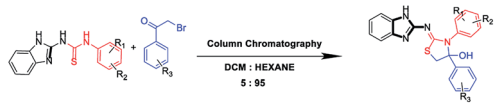
As the retention factor (R_f-value) of thiourea **1** was low, it was moving slowly and R_f-value of **2a** was higher than thiourea **1**, which was moving fast. Therefore, the height of the second layer of silica adsorbed by thiourea **1** should be long enough such that **2a** will react with it and should not elute without reaction.

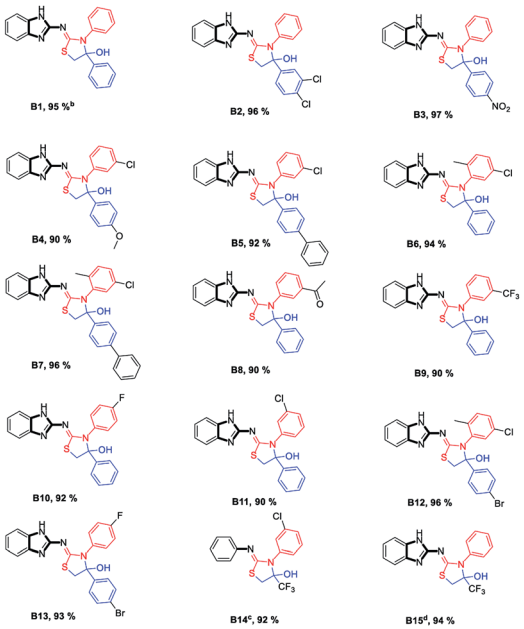
We explored the scope of this reaction with respect to the substituents. We examined by choosing a synthesis of benzimidazol-2-imino-thiazolidine-4-ol **B1** as a model reaction for optimization (Table 1, **B1**), the reaction of benzimidazol-phenyl-thiourea **1** and 2-bromoacetophenone **2a** in column at room temperature using 5% DCM : hexane as a solvent for column chromatography. The eluent from the column was collected in ice cold hexane. The stabilized solid product was afforded with high yield (95%, Table 1) of benzimidazole-2-imino-thiazolidine-4-ol **B1**. In the electronic study of the substituent, the electron withdrawing group on 2-bromoacetophenone led to a high yield compared to electron donating groups (Table 1). The efficiency of this protocol was successfully demonstrated by the reaction of thiourea **1** and 3-bromo-1,1,1-trifluoropropan-2-one (Table 1, **B14** and **B15**), although with a high yield (96 and 98%). The reaction was sluggish for donating and fusing ring 2-((1*H*-benzo[*d*]imidazol-2-yl)imino)-4-(naphthalen-2-yl)-3-phenylthiazolidin-4-ol. This method provides easy access for synthesizing hitherto unknown benzimidazole-biphenyl-thiazolidin-4-ol and benzimidazole-phenyl-trifluoro methyl-thiazolidin-4-ol compounds (**B1–B13** and **B15**).

The genesis of mechanism depends on the pK_a values of amines,[20] *i.e.*, the basicity of respective nitrogen of amines (Scheme 2). Obviously, the nitrogen of benzimidazole is more basic than aniline; thus, benzimidazole nitrogen was converted to imine by attacking the 2-bromoacetophenone through sulfur. In the next step, aniline nitrogen of thiourea underwent cyclization by attacking carbonyl carbon and formed a benzimidazol-diphenyl-2-imino-thiazolidine-4-ol (**B1**). Thus, the stability of product was maintained by the four bond distant benzimidazole ring as compared to hemiaminal **Aa**.

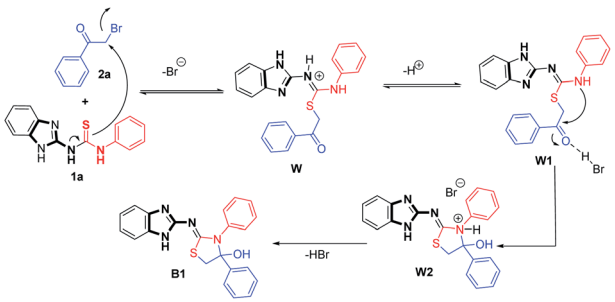
In terms of importance of these compounds, curiously we observed that the anticancer activities of benzimidazol-diphenyl-2-imino-thiazolidine-4-ols are really promising. Though these moieties are smaller in size, their anticancer activities are attractive than other hemiaminals prepared by

Table 1 Scope of reaction for the synthesis of product B[a]





[a] Reaction condition: benzimidazol-phenyl thiourea **1a** (1 mmol) and phenacyl bromide **2a** (1 mmol), under column chromatography using (5 : 95) (DCM : hexane) solvent at room temperature. [b] Yields are reported after solidification. [c] **B14** prepared from diphenyl thiourea and trifluoro methane 2-bromoacetophenone. [d] **B15** prepared from **1a** and trifluoro methane 2-bromoacetophenone.



Scheme 2 Plausible mechanism for the synthesis of product **B1**.

earlier methods. All products were screened against the human colon, prostate, lung and breast cancer cell lines by using doxorubicin as a standard. Average values of three individual experiments were recorded by 50% inhibitory concentration after 48 h of actual drug treatment. It is again worthy to note that fluorinating phenyl rings are exclusively effective against lung cancer than trifluoro methylating phenyl rings on benzimidazol-diphenyl-2-imino-thiazolidine-4-ol. Thus, by the collected data, products **B9** (IC_{50} = 3.890 μM) and **B10** (IC_{50} = 2.798 μM) and **B13** (IC_{50} = 3.140 μM) have been extensively studied. It is again important to point out that due to the increase in the size of this thiazolidin-4-ol by bulky substitution on phenyl rings, there is a continuous decrease in the anticancer activity of the respective compound. Thus, only fluorination or a small size benzimidazol-diphenyl-2-imino-thiazolidine-4-ol is efficient for its anticancer activity.

Conclusions

In conclusion, the chemistry of the synthesis of hemiaminal products is really challenging. We have proposed the synthesis of hemiaminals, benzimidazol-diphenyl-2-imino-thiazolidine-4-ol by column chromatography. The method is metal free, catalyst free and highly efficient. The stability of hemiaminal has been increased by the insertion of four bond distant benzimidazole ring. We have escaped the process of extraction and protected the stability of product. Simultaneously, we performed reaction, separation and solidification in a single unit. The method is purely efficient, ecofriendly and sustainable for a green approach. The benzimidazol-diphenyl-2-imino-thiazolidine-4-ol products were screened against four human colon, breast, lung and prostate cancer cell lines with respect to doxorubicin as a standard. We are happy to mention that fluorinating phenyl rings in benzimidazol-diphenyl-2-imino-thiazolidine-4-ol enhance anticancer activity, which is hitherto unknown for lung cancer. Increasing the activity of these types of compounds is going on in our laboratory.

Experimental section

Reactions were performed in oven-dried borosil glassware. Commercial reagents were purchased from Sigma Aldrich, Fluka or Alfa Aesar and used as-received. Anhydrous solvents were purchased from Rankem. All solvents were distilled before use. Thin layer chromatography (TLC) was performed using precoated glass silica gel plates and visualized by UV fluorescence of 254 nm short wavelength ultraviolet light. TLC was exposed to iodine vapours and a solution of *p*-anisaldehyde (3 g of *p*-anisaldehyde, 1 mL of AcOH and 3 mL of concentrated H_2SO_4 in 125 mL of HPLC grade MeOH) followed by direct heating on hot plate. Melting points were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 300 MHz, 500 MHz and 700 MHz NMR spectrometer. For ^{1}H NMR, chemical shifts (δ) were expressed in parts per million (ppm) with respect to TMS as internal standards (0.00 ppm) and $CDCl_3$ (δ = 77.0)/DMSO-d_6 (39.43) used as internal standards for ^{13}C NMR. Coupling constants (J) were expressed in Hertz (Hz). Mass spectra were recorded by using 70 eV spectrometer.

General procedure for the preparation of product benzimidazol-2-imino-thiazolidine-4-ol B1

Benzimidazol-phenyl-thiourea **1a** (1 mmol, 268 mg) was dissolved in minimum THF and 2-bromoacetophenone **2a** (1 mmol, 199 mg) was dissolved in minimum DCM at room

temperature, and both the components adsorbed separately on 60–120 mess silica gel using vacuum pump.

The glass column of 100 cm length was prepared by five different silica layers. After cotton plug at bottom near to stopcock, the first layer was packed by dry silica up to 05 cm for the good separation. Following the first layer, the second layer was packed by adsorbed silica of thiourea **1a** (should be long layer, up to 30–40 cm long). The third layer was packed by dry silica gel up to 1–2 cm long. The fourth layer was packed with silica adsorbed by **2a** up to 05 cm long and this fourth layer covered by 1–2 cm long dry silica followed by cotton plug to stabilize silica layer. The reaction was performed in column using 5% (DCM : hexane) as a solvent for column chromatography. The eluent from column was collected in ice cold hexane. After some time, white precipitate started to appear in cold hexane, and the elution of column was continued without any break using 5% (DCM : hexane) as a solvent. The beakers with precipitated product were maintained at ice cold temperature. When the white precipitate stopped to appear, we have closed the stopcock to finish the reaction. The solid precipitate was filtered out using vacuum pump. The filtrate was evaporated for slow evaporation of DCM, and the solid product was precipitated again in hexane which was filtered out using vacuum pump. The dried solid benzimidazole-2-imino-thiazolidine-4-ol **B1** product was afforded with high yield (95%). The product **B1** was characterized by ^{1}H-NMR, ^{13}C-NMR and ESI mass-HRMS.

Traditional method. 1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-phenylthiourea **1a** (1 mmol, 268 mg) and 2-bromoacetophenone **2a** (1 mmol, 199 mg) were dissolved separately in 2 mL THF each. Both the solutions were mixed at room temperature with constant stirring. Within a minute the turbidity appeared with completion of reaction which was monitored by TLC. To our surprise, reaction completed with a high rate of reaction. The reaction was extracted by ethyl acetate and separated by column chromatography. At the time of separation by column chromatography, we found that the original product started to decompose. With precise observation, the product decomposed from the extraction step itself. As we could not get pure product, we could not report the yield of pure product. The rate of decomposition was also very high. Thus, it was confirmed that by traditional method, we can synthesize the product benzimidazole-2-imino-thiazolidine-4-ol **B1** but it is very difficult to purify and characterize with purity.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

H. M. M. is thankful to Director, IICT, for continued support and encouragement. V. M. B. is thankful to the Director Institute of Science and P. R. M. is thankful to the CSIR, New Delhi, for a fellowship.

Notes and references

1 S. V. Ley, I. R. Baxendale, R. N. Bream, P. S. Jacson, A. G. Leach, D. A. Longbottom, M. Nesi, J. S. Scott, R. I. Storer and S. J. taylor, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3815–4195.
2 A. M. Hafez, A. E. Taggi, T. Dudding and T. Lectka, *J. Am. Chem. Soc.*, 2001, **123**, 10853–10859.
3 S. C. Dakdouki, D. Villemin and N. Bar, *Eur. J. Org. Chem.*, 2010, 333–337.
4 G. Strohlein, Y. Assuncao, N. Dube, A. Bardow, M. Mazzotti and M. Morbidelli, *Chem. Eng. Sci.*, 2006, **61**, 5296–5306.
5 O. Trapp, S. K. Weber, S. Bauch and W. Hafstadt, *Angew. Chem., Int. Ed.*, 2007, **14**, 7307–7310.
6 M. Ejlersen, C. Lou, Y. S. Sanghvi, Y. Tor and J. Wengel, *Chem. Commun.*, 2018, **54**, 8003–8006.
7 V. Chiroli, M. Benaglia, F. Cozzi, A. Puglisi, R. Annunziata and G. Celentano, *Org. Lett.*, 2013, **15**, 3590–3593.
8 A. M. Hafez, A. E Taggi and T. Lectka, *Chem.–Eur. J.*, 2002, **8**, 4114–4119.
9 S. T. Tuncel and I. Dogan, *Chirality*, 2020, **32**, 866–875.
10 S. E. Gunal, G. S. Gurses, S. S. Erdem and I. Dogan, *Tetrahedron*, 2016, **72**, 2122–2131.
11 K. Tanaka, K. Nomura, H. Oda, S. Yoshida and K. Mitsuhashi, *J. Heterocycl. Chem.*, 1991, **28**, 907–911.
12 (*a*) V. S. Karavan and V. A. Nikiforov, *Russ. J. Org. Chem.*, 1999, **35**, 741–747; (*b*) M. N. Murawiewa and V. M. Schtschukina, *J. Gen. Chem. USSR (Engl. Transl.)*, 1960, **30**, 2327–2331.
13 T. Dalmal, K. Appalanaidu, U. B. Kosurkar, N. J. Babu and R. M. Kumbahre, *Eur. J. Org. Chem.*, 2014, 2468–2479.
14 (*a*) T. Iwasawa, R. J. Hooley and J. Rebek, *Science*, 2007, **317**, 493–496; (*b*) R. J. Hooley, P. Restorp, T. Iwasawa and J. Rebek, *J. Am. Chem. Soc.*, 2007, **129**, 15639–15643.
15 P. Subik, B. Welc, B. Wisz and S. Wolowiec, *Tetrahedron Lett.*, 2009, **50**, 6512–6514.
16 M. Barys, Z. Ciunik, K. Drabent and A. Kwiecien, *New J. Chem.*, 2010, **34**, 2605–2611.
17 (*a*) *WHO Model Formulary*, ed. M. C. Stuart, M. Kouimtzi and S. R. Hill, 2008, 94–96, ISBN 9789241547659; (*b*) World Health Organization, *Model list of essential medicines*, 2019, Licence: CCBY-NC-SA 3.0 IGO.
18 N. Karaali, S. Aydin, N. Baltas and E. Mentese, *J. Heterocycl. Chem.*, 2020, 1–10.
19 A. Ozdemir, S. Turanh, b. Cahskn, M. Arka and E. Banoglu, *Pharm. Chem. J.*, 2020, **53**, 1036–1046.
20 S. Murru, C. B. Singh, V. Kavala and B. K. Patel, *Tetrahedron*, 2008, **64**, 1931–1942.
21 CCDC: 1041069 contains crystal data with structure refinement data as ESI† for this paper. These data can be obtained at https://summary.ccdc.cam.ac.uk/structure-summary-form or by writing to the Cambridge Crystallographic Data Centre, Cambridge CB2 1EZ, UK, E-mail: deposit@ccdc.cam.ac.uk.