RSC Advances

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Advances*, 2013, **3**, 3407

An efficient stereo-controlled synthesis of bis-pyrimido-[4,5-*d*]-pyrimidine derivatives *via* aza-Diels–Alder methodology and their preliminary bioactivity†‡

Subrata Das,*[ab] Ashim Jyoti Thakur,*[b] Tapas Medhi[c] and Babulal Das[d]

Received 9th September 2012,
Accepted 30th October 2012

DOI: 10.1039/c3ra22089c

www.rsc.org/advances

The one-pot synthesis of novel fused bis-pyrimido-[4,5-*d*]pyrimidine derivatives by a three-component reaction of 6-[(dimethylamino)methylene]1,3-dimethylaminouracil, terephthalaldehyde and amino derivatives has been depicted. The structures of the compounds were established by studying various spectroscopic methods and single-crystal X-ray crystallography. The long range *W*-coupling constant in ^{1}H-NMR spectra is an infrequent example, where our synthesized novel compounds show such a distinctive constant. The synthetic strategy provides an efficient way to synthesise bis-pyrimidine-fused heterocycles that can be explored for further potential pharmaceutical or biological activities.

Introduction

The Diels–Alder reaction (DAR) is one of the most well studied methods for the synthesis of cycloaddition adducts in organic transformation. The corresponding aza version of the DAR, *i.e.* aza-Diels–Alder reaction (aza-DAR) is a powerful methodology for the construction of 100% economically nitrogen-containing six-member ring compounds in a single step and constitute a broad spectrum of natural products, alkaloids, pharmaceutically and biologically active compounds and polymeric materials.[1] The regio- and stereo-selectivities of the products *via* the DAR methodology are well known under mild reaction conditions.[2] Not only have the chemical synthesis and the total synthesis of bio-active compounds been reported *via* aza-DAR, but the biosynthesis of many natural products has also been reported.[3]

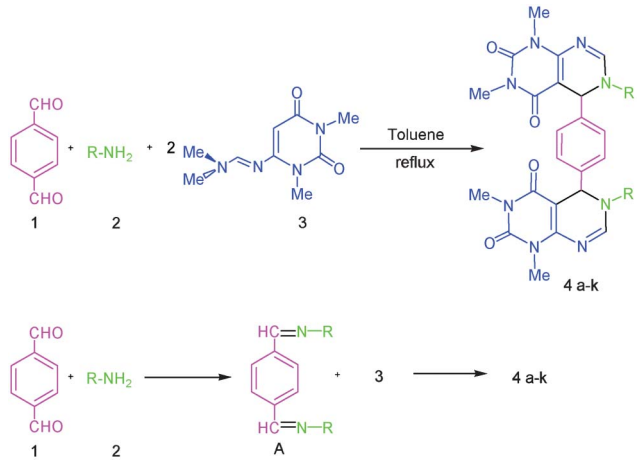
The existing compelling evidence of aza-dienes having low reactivity in normal DARs has been based on calculated energies of activation (E_a). Experimental ionization potentials, electron affinities and theoretical energies of the HOMO and LUMO orbitals for a number of aza-dienes have been reported.[4] One of the reasons for the low reactivity of aza-dienes is the presence of the nitrogen atom that creates a π-electron-deficient system and thus lowers the reactivity in normal $HOMO_{diene}$ controlled DARs with electron poor dienophiles.[5] It has also been postulated that the dienophilic nature of the pyrimidine ring is rather limited, and the diene properties of vinylpyrimidines have not yet been established.

Pyrimidine and fused pyrimidine derivatives are indispensable components of the biopolymers of RNA/DNA and are well known for their bioactivities. In 1960 Taylor *et al.* first reported the synthetic procedure for a series of pyrimido[4,5-*d*]pyrimidines as potential diuretic agents starting with 4-amino-5-cyanopyrimidines and 4-aminopyrimidine-5-carboxmides.[6] Sharma *et al.* reported the synthesis of pyrimido[4,5-*d*]pyrimidines and studied their bioactivity.[7] Saravanan *et al.* also reported that pyrimido[4,5-*d*]pyrimidine derivatives act as nucleoside transport inhibitors (NTI) with improved *in vivo* pharmacokinetic properties and reduced α_1-acid glycoprotein (AGP) binding relative to dipyridamole.[8] Recently pyrimido[4,5-*d*]pyrimidine derivatives have exposed good potentiality against *in vitro* HBV DNA replication inhibition and as nucleoside transport inhibitors.[9] We have previously shown 6-[(dimethylamino)methylene]-1,3-dimethylaminouracil (amidine) as an interesting reagent for novel fused pyrimidine derivatives.[10] Here, we have described aza-DARs of one pot three component reactions of terephthalaldehyde (**1**), amines (**2**) and amidine (**3**), which lead to the production of novel bis-pyrimido[4,5-*d*]pyrimidine derivatives (Scheme 1). To the best of our knowledge, there are no reports in the literature for the synthesis of such bis-pyrimido-[4,5-*d*]pyrimidine derivatives *via* our developed methodology and no studies of their bioactivity.

[a]*Department of Chemistry, National Institute of Technology-Agartala, Tripura, India. E-mail: subrataorg@gmail.com; Tel: 919612176571*
[b]*Department of Chemical Sciences, Tezpur University, Assam, India*
[c]*Department of Molecular Biology and Biotechnology, Tezpur University, Assam, India*
[d]*Indian Institute of Technology-Guwahati, Department of Chemistry, Guwahati-781039, Assam, India*

† Crystallographic data (excluding structure factors) for **4b** have been deposited with the Cambridge Crystallographic Data Centre. CCDC reference number 878092. For crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ra22089c

‡ Crystal data for **4b** ($C_{36}H_{35}Cl_2N_{10}O_5$): M_r = 758.64, orthorhombic, Pnma, a = 16.0317(17) Å, b = 22.181(2) Å, c = 10.2433(13) Å, V = 3642.5(7) Å^3, T = 296(2)K, Z = 4, R_{int} = 0.1134, reflection collected/unique = 35187/3857, $2\theta_{max}$ = 53.0°, GOF = 1.070, $R_1[I > 2\sigma(I)]$ = 0.0909 and wR_2 = 0.2956 (for all data).



Scheme 1 Synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivatives (**4a–k**).

Results and discussion

Chemical synthesis

Two major approaches were applied to the synthesis of the proposed pyrimidine compounds. The first approach employed the corresponding bis-pyrimido-[4,5-*d*]pyrimidine derivatives *via* the one-pot multi-component reaction of amidine (**3**), terephthalaldehyde (**1**) and aniline (**2a**) under reflux in toluene without using any catalyst. The second one involved the construction of a one-pot multi-component reaction using $In(OTf)_3$ as catalyst in chloroform for a successive three step reaction. Initially, we intended to adopt the direct DAR through reflux in nitrobenzene aromatization. However, it was very complex to get bis-pyrimido-[4,5-*d*]pyrimidine up to a good quantity (see Table 1). In those cases some product also got burnt at the time of removing the nitrobenzene from the reaction mixture. To increase the reaction yield with more trustworthy reaction conditions, we used various reaction directions (Table 1). In our first approach we found that toluene is a good solvent for the synthesis of bis-pyrimido-[4,5-*d*]pyrimidine (see entry 10, Table 1).

Table 1 Optimization of the reaction conditions for the model reactions between (**1**), (**2a**) and (**3**)

Entry	Reaction Conditions	Time/h	Yield (%)[a]
1	Water, reflux	12	No product
2	Water, reflux, 15 mol% *p*-TSA	12	No product
3	H_2O, SDS[b], rt, 10 mol% *p*-TSA	12	No product
4	H_2O, SDS, reflux, 10 mol% *p*-TSA	12	Trace
5	CH_3CN, reflux	12	Trace
6	EtOH, reflux	12	No product
7	MeOH, reflux	12	No product
8	DMF, reflux	12	20
9	$PhNO_2$, reflux	12	44
10	Toluene, reflux	12	91

[a] Yield (%) is referred to isolated yields and calculated from mol of product/mol of initial substrate $\times 100$. [b] SDS = Sodium dodecyl sulphate.

Table 2 Synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivatives (**4a–k**)[a]

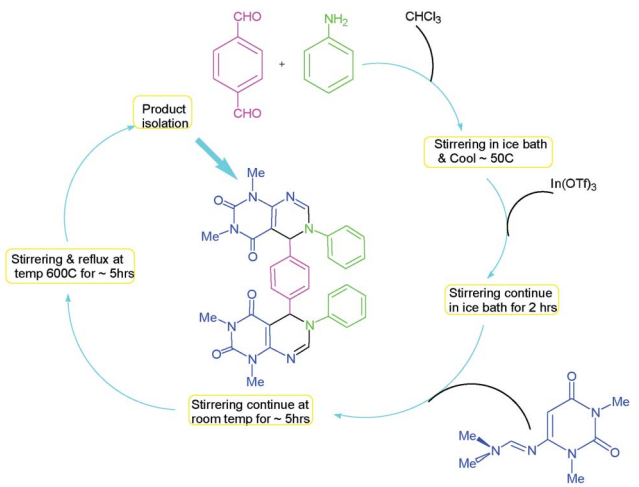
Entry	R	Time/h	m.p. (°C)	Yield (%)
a	C_6H_5	11	360	91
b	*p*-Cl–C_6H_4	10	365	97
c	*p*-OCH_3–C_6H_4	12	276	73
d	*p*-CH_3–C_6H_4	11	293	81
e	*o*-CH_3–C_6H_4	11	295	78
f	*p*-NO_2–C_6H_4	12	287	83
g	*o*-NO_2–C_6H_4	12	229	81
h	*m*-NO_2–C_6H_4	12	287	84
i	CH_3	24	—	21
j	CH_3–$(CH_2)_3$	24	—	17
k	C_6H_4–CH_2	24	—	25

[a] Isolated yield.

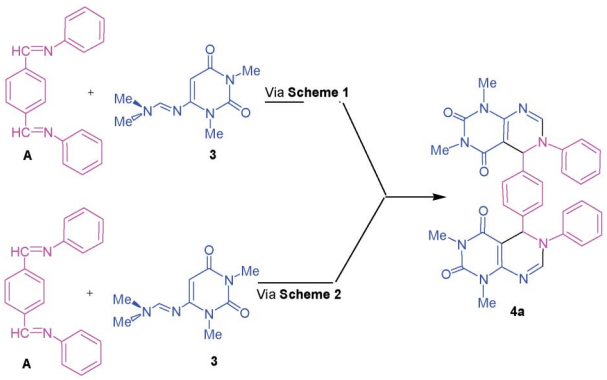
Based on this optimised reaction strategy we continued our generalization with a series of aromatic and aliphatic amines (entries **4a–k**, Table 2) (Scheme 1). Aromatic amines led to the formation of more stereo-controlled bis-pyrimido-[4,5-*d*]pyrimidine derivatives with high yields as compared to aliphatic ones. The results are summarized in Table 2. Here, the reaction proceeds in two steps, first presumably *via* the formation of condensation reaction of (**1**) and (**2a**) to form bis-imine (**A**) (Scheme 1). Second step is the formation of hetero-Diels–Alder product of (**A**) with (**3**) to form bis-pyrimido[4,5-*d*]pyrimidine (**4a**).

Amine moieties having chloro-substituents lead to the production of good yields followed by unsubstituted aromatic amines. In the case of aliphatic amines we observed very poor yields (entries **i–k**, Table 2). In our second approach we did not observe any spectacular results as compared to the first approach. Furthermore, in the second approach the reaction proceeded in the presence of hygroscopic catalyst followed by complex reaction conditions (Scheme 2). Scheme 1 is more economical and sympathetic than Scheme 2 in context with synthetic inspection.

To justify the reaction procedure we also tried a two step reaction for the synthesis of bis-pyrimido[4,5-*d*]pyrimidine



Scheme 2 Synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivative (**4a**).



Scheme 3 Synthesis of bis-pyrimido[4,5-*d*]pyrimidine derivative (**4a**).

(**4a**). For that we first synthesized the bis-imine (**A**, R = Ph), by the reaction of (**1**) and (**2a**). Then in the second step we carried out the reaction of the bis-imine (**A**) with the amidine (**3**) (Scheme 3) *via* both the reaction approaches. We achieved the same product (**4a**) without observing any customary results.

In the ^{1}H NMR spectrum of **4a** (R = Ph), the peaks at δ = 3.25 (6H) and 3.31 (6H) ppm are due to the six *N*-methyl groups, the peak at δ = 5.99 ppm is due to the CH proton, the peaks within δ = 7.03–7.06 and δ = 7.27–7.38 ppm are due to the five aromatic protons and the peak at δ = 7.78 ppm is due to the imine proton. Similarly, (**4b**) was obtained in 97% yield within 10 h. In the ^{1}H NMR spectrum of **4b** (R = ClPh), the peaks at δ 3.27, 3.54 and 5.96 (J = 10.8 Hz, CH), 6.98–7.00, 7.28–7.37 and 7.73 (J = 7.8 Hz) ppm indicate the formation of the corresponding chloro-subtituted bis-pyrimido[4,5-*d*]pyrimidine derivative (Fig. 1).

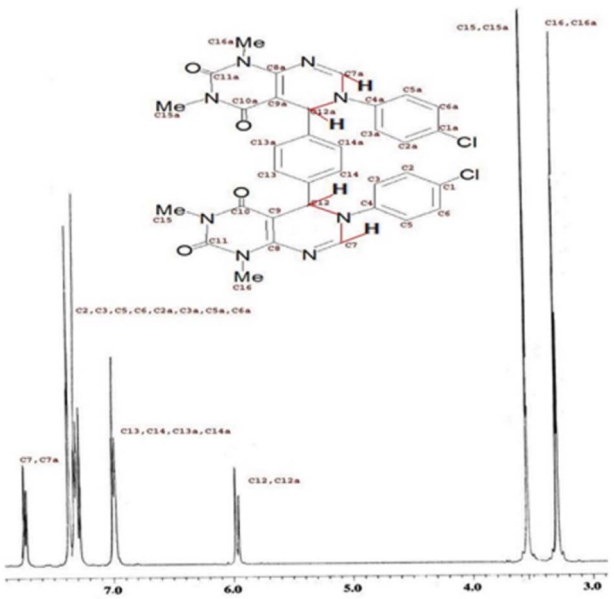


Fig. 1 The ^{1}H NMR spectrum of **4b**, which shows the long range *W*-coupling constant.

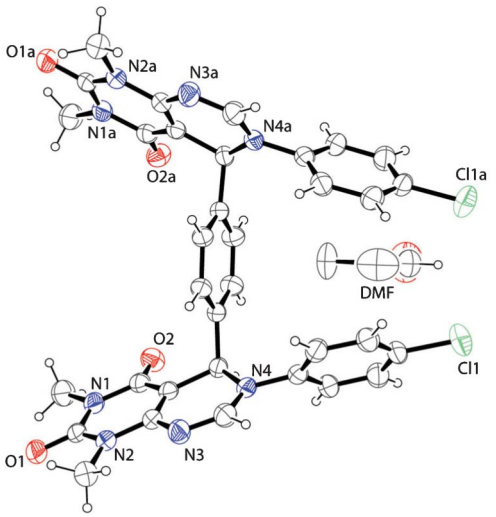


Fig. 2 The ORTEP diagram of compound **4b** (thermal ellipsoids are drawn with 30% probability). The letter "a" in the atom labels indicates that these atoms are at equivalent positions (x, 1/2−y, z).

The value of the *W*-coupling constant is the exhibited difference due to the substituent proximity in relation to the *W*-positioned hydrogen atoms. If the substituents are on the same side of the ring as the *W*-positioned hydrogen atoms, low *W*-coupling constants are observed, while substituents on the opposite side of the *W*-positioned hydrogen atoms seem to permit higher coupling constants.[11] The structure was further confirmed through single crystal X-ray analysis and the long range *W*-coupling constant was also supported by X-ray analysis. Suitable crystals were obtained by the slow evaporation of **4b** from DMF solution. The ORTEP diagram of the compound **4b** is shown in Fig. 2 and it was found that the compound had mirror symmetry. From the ORTEP diagram we can suggest that compound **4b** may be in the *cis* form due to same priority groups (in this case H and the lone pair on nitrogen) being on the same side across the C=N bond. This *cis* conformation may be further supported by NMR data, *i.e.* in the NMR spectrum we saw a low coupling constant value. This *cis* form may also be stabilised by the DMF solvent molecule.

In the lattice the carbonyl group of the compound exhibits a large number of C–H···O interactions with the adjacent molecule. The C=O group of DMF also interacts with the chloro-benzene ring of the compound *via* similar weak interactions, further contributing to the stability of the molecule.

A plausible mechanism for the reaction is described in the Fig. 3. Mechanistically, in the three-component reaction, the bis-imine (**A**) is first formed by the reaction between the dialdehyde (**1**) and amine (**2a**). Bis-imine (**A**) acts as a dienophlie which reacts with amidine (**3**) diene to form the product (**4a**) *via* the normal hetero-Diels–Alder reaction.

Antimicrobial activity

All the newly synthesized bis-pyrimido[4,5-*d*]pyrimidine derivatives were assessed for their *in vitro* antibacterial properties against both gram positive and gram negative bacteria *viz.*

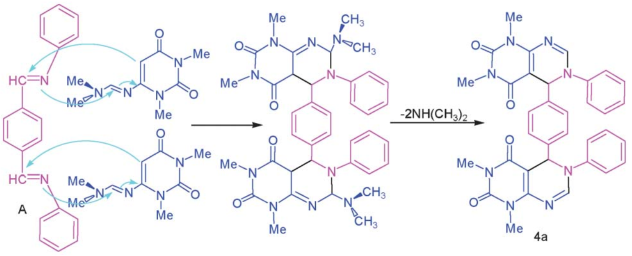


Fig. 3 The plausible reaction mechanism.

Staphylococcus aureus (ATCC 11632), *Bacillus subtilis* (ATCC 11774), *Escherichia coli* (ATCC 13607), and *P. diminuta* (MTCC 7814) and antifungal activity against *A. niger* (MTCC 284) and *Candida albicans* (MTCC 227).

Nutrient agar and sabouraud dextrose agar were employed for bacterial and fungal growth, respectively. An organized form of the data is illustrated in Table 3, Table 4 and Table 5 which reveals that the antibacterial behaviour of the tested compounds are highly significant and pronounced compared to their antifungal behaviour. Gram negative bacteria were found to be sensitive to the bis-pyrimido[4,5-*d*]pyrimidine derivatives. On the other hand, bis-pyrimido[4,5-*d*]pyrimidine derivatives showed the least growth inhibition against fungi. Furthermore, an elaborate examination of the screening results reveals that all the bis-pyrimido[4,5-*d*]pyrimidine derivatives equally showed good anti-bacterial properties, only *p*-Cl substituted bis-pyrimido[4,5-*d*]pyrimidine derivatives (MIC 12.5 μg) show good activity compared to the other bis-pyrimido[4,5-*d*]pyrimidine derivatives (MIC 25–50 μg).

The agar well diffusion technique was used in the present investigation, following the procedure described by Boakye-Yiadom,[12] Banso and Adeyemo[13] and Radhika, *et al.*[14] 200 μl of overnight grown culture (inoculums size was 10^7–10^8 cells mL^{-1} as per McFarland standard) of the test bacterial strains were seeded on the surface of the Muller Hinton agar while the fungal strains (0.5–2.5 × 10^6 mL^{-1}) were seeded on the sabouraud dextrose agar (SDA) medium, using a sterile glass pipette and spread over the medium using a sterile glass spreader. A total of five wells, 6 mm each were made on the solidified nutrient agar (NA) and sabouraud dextrose agar (SDA) plates, respectively, with the help of a sterile cork borer. Concentrations of 0.125 mg mL^{-1}, 0.25 mg mL^{-1}, 0.5 mg mL^{-1}, 1.0 mg mL^{-1} and 2.0 mg mL^{-1} of the synthesized compound dissolved in sterilized DMSO were introduced into each of the wells. Due to the poor solubility of bis-pyrimido[4,5-*d*]pyrimidine derivatives in common organic solvents or water, all the compounds were dissolved and tested for corresponding antimicrobial activities in 10% DMSO (v/v) aqueous solution as DMSO solvent is highly miscible with water, highly polar and stable with exceptional solvent properties.[15] It was also found from literature that 10% DMSO (v/v) has been used for antimicrobial and other biological activities.[16] As in our previous study of the antimicrobial activities of bis-pyrimido[4,5-*d*]pyrimidine derivatives we first screened the percentage of DMSO (ranging from 1% to 15%, v/v) that is tolerated by cells or not. In the presence of 10% DMSO (v/v) both the gram positive and gram negative bacteria had no detectable effect on bacterial growth. Again it was also noticeable that different organisms responded differently to the same concentration of a given solvent.[17] Here, our tested bacteria and fungi were comfortable with 10% DMSO (v/v) and it showed no detectable effect on growth. Compounds at concentrations of 125 μg–2000 μg mL^{-1} were prepared in 10% DMSO (v/v). Streptomycin (1 mg ml^{-1}) and sterilized 10% DMSO (v/v) without the synthesized compound were taken as a positive and negative control, respectively, and for fungi clotrimazole was used to serve as a positive control. Plates with discs containing respective 10% DMSO (v/v) solvents also served as controls. It was found that

Table 3 Antibacterial activity tests of the bis-pyrimido[4,5-*d*]pyrimidine derivatives (**4a–h**)[a]

Entry	Gram Negative Bacteria		Gram Positive Bacteria		Fungi	
	EC	*PD*	*BS*	*SA*	*CA*	*AN*
4a	+	+	−	−	−	−
4b	+	+	−	−	−	−
4c	+	+	−	−	−	−
4d	+	+	−	−	−	−
4e	+	+	−	−	−	−
4f	+	+	−	−	−	−
4g	+	+	−	−	−	−
4h	+	+	−	−	−	−

[a] *EC = Escherichia coli*; *PD = P. diminuta*; *BS = Bacillus subtilis*; *SA = Staphylococcus aureus*; *CA = Candida albicans*; *AN = A. niger*.

Table 4 Zone of inhibition of the bis-pyrimido[4,5-*d*]pyrimidine derivatives (**4a–h**)

Entry	Zone of Inhibition/mm			
	E. coli		*P. diminuta*	
4a	11	10	11	11
4b	13	12	11	11
4c	12	11	13	13
4d	11	11	12	11
4e	11	11	13	13
4f	11	11	13	12
4g	12	13	13	13
4h	11	11	11	11

Table 5 MIC values of the pyrimido[4,5-*d*]pyrimidine derivatives (**4a–h**)

Entry	MIC (mg ml^{-1})	
	E. coli	*P. diminuta*
4a	0.5	0.25
4b	0.125	0.25
4c	0.25	0.25
4d	0.25	0.25
4e	0.25	0.25
4f	0.25	0.5
4g	0.125	0.25
4h	0.25	0.5

DMSO showed no effect against the microorganisms under test. In the case of bacteria, the plates were incubated at 37 ± 2 °C for 24 h while for fungal cultures; the plates were kept at 27 ± 2 °C for 48 h. The observed zones of inhibition were measured using a transparent metric ruler. The experiment was done thrice and the mean values were determined.

The minimum inhibitory concentration (MIC) of the synthesized compounds was determined by the micro dilution method and compared to broad-spectrum antibiotics. MIC is defined as the lowest concentration of a compound that inhibits visible growth of the test organism. All the synthesized compounds exhibited remarkable *in vitro* antibacterial activity against the tested bacteria only and were less significant towards fungal strains compared to the reference antibiotics.

Conclusions

Diels–Alder methodology (aza-version) has been found to be a useful and efficient method for the construction of fused heterocyclic systems like bis-pyrimido[4,5-*d*]pyrimidine derivatives in good to excellent yields. The scope and generality of this methodology have been successfully demonstrated by synthesizing a variety of fused pyrimidine derivatives. We believe that our developed methodologies will open a new vista for the construction of wide varieties of synthetic/natural/biological compounds. The prominent advantages of the described report are the novelty of synthesized compounds, operational simplicity, good yields and easy workup procedures without the need for any further separation techniques (for Scheme 1). Preliminary antimicrobial activity tests show that bis-pyrimido[4,5-*d*]pyrimidine derivatives are active against gram negative bacteria and inactive against gram positive bacteria.

Experimental section

Melting points were determined with Büchi 504 apparatus. IR spectra were recorded as KBr pellets with a Nicolet (Impact 410) FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a JNM ECS 400 MHz NMR spectrophotometer (JEOL) using tetramethylsilane (TMS) as the internal standard. X-Ray intensity data were collected with a Bruker SMART APEX CCD area-detector diffractometer with Mo-Kα radiation (λ = 0.71073 Å). The structures were solved by SHELX97 and refined by full-matrix least-squares on F^2 (SHELX97). Reactions were monitored by thin-layer chromatography using aluminium sheets with silica gel $60F_{254}$ (Merck). Elemental analyses were carried out with a Perkin-Elmer CHN analyzer (2400 series II). Mass spectra were recorded with a Waters Q-TOF Premier and Aquity UPLC spectrometer. All the chemicals were used as received.

Typical reaction procedures for the synthesis of pyrimido[4,5-*d*]pyrimidine derivative 4a

For Scheme 1. A mixture of 6-[(dimethylamino)methylene]1,3-dimethylaminouracil (**3**) (209 mg, 1 mmol), terephthalaldehyde (**1**) (134 mg, 0.5 mmol) and aniline (**2a**) (0.091 ml, 1 mmol) was placed in a 50 ml round bottom flask, then ~15 ml toluene was added and it was allowed to reflux overnight. After completion of the reaction the product precipitated out from the reaction mixture which was then washed with ethyl acetate to remove any unreacted reactant and other byproducts. The pure product was dried at high vacuum using a pump and solubilised in DMF, re-crystallized and we got the bright yellow pure product (**4a**) in 91% yield; m.p. 360 °C.

The same reaction procedure was followed for the rest of the substrates.

For Scheme 2. 6-[(Dimethylamino)methylene]1,3-dimethylaminouracil (**3**) (209 mg, 1 mmol), terephthalaldehyde (**1**) (134 mg, 0.5 mmol) and aniline (**2a**) (0.091 ml, 1 mmol) was mixed in dry chloroform (20 mL) in a 50 mL round-bottomed flask and was stirred at room temperature for 10 min. The reaction mixture was cooled to 0 °C using an ice bath, then $In(OTf)_3$ (5.6 mg, 10% of 1 mmol) was added with constant stirring. Gradually, the reaction mixture was allowed to come to room temperature. Then the reaction mixture was refluxed at 60 °C with constant stirring and after 6 h we obtained the pure product (**4a**) in 91% yield. The product was separated through TLC, dissolved in distilled DMF and then warmed, filtered, allowed to cool and the filtrate was evaporated at room temperature for crystallization.

To improve the solubility of the compounds, the pure compounds were refluxed in nitrobenzene for 5/6 h.

The same reaction procedure was followed for the rest of the substrates.

Pure compounds were insoluble in water, sparingly soluble in common organic solvents and completely soluble in aprotic polar solvents like DMF, DMSO and DMAc *etc*.

5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,4-dioxo-6-phenylpyrimido[4,5-*d*]-pyrimidin-5-yl)phenyl)-1,3-dimethyl-6-phenylpyrimido[4,5-*d*]pyrimidine-2,4-(1*H*,3*H*)-dione (4a)

IR (KBr) (ν_{max}/cm^{-1}) 3073.78, 2941.61, 1698.17, 1640.16, 1532.89, 1471.29; δ_H (100 MHz; DMSO-d_6) 3.25 (s, 6H, NCH_3), 3.31 (s, 6H, NCH_3), 5.99 (d, 2H, J = 10.52 Hz, CH), 7.03–7.06 (m, 4H, arom.), 7.27–7.38 (m, 8H, arom.), 7.78 (d, 2H, J = 7.8 Hz, CH=N); δ_C (100 MHz; DMSO-d_6) 28.2, 29.7, 58.5, 58.7, 91.1, 123.7, 123.8, 127.1, 127.2, 130.1, 130.2, 133.5, 140.1, 141.9, 142.0, 148.9, 152.0, 152.1, 161.1; MS, m/z 614 (M^+); Anal. Calcd (%) for $C_{34}H_{30}N_8O_4$: C, 66.44; H, 4.92; N, 18.23. Found C, 66.43; H, 4.96; N, 18.21.

6-(4-chlorophenyl)-5-(4-(6-(4-chlorophenyl)-1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,4-dioxopyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-5,6-dihydro-1,3-dimethylpyrimido-[4,5-*d*]-pyrimidine-2,4(1*H*,3*H*)-dione (4b)

IR (KBr) (ν_{max}/cm^{-1}) 3071.98, 2943.13, 1698.27, 1641.06, 1533.19, 1471.27; δ_H (100 MHz; DMSO-d_6) 3.27 (s, 6H, NCH_3), 3.54 (s, 6H, NCH_3), 5.96 (d, 2H, J = 10.8 Hz, CH), 6.98–7.00 (m, 4H, arom.), 7.28–7.37 (m, 8H, arom.), 7.73 (d, 2H, J = 7.8 Hz, CH=N); δ_C (100 MHz; DMSO-d_6) 28.7, 29.9, 58.4, 58.6, 91.0, 122.9, 123.4, 126.9, 127.0, 129.7, 129.8, 133.6, 140.1, 141.7, 142.2, 148.7, 152.7, 153.1, 161.7; MS, m/z 682 (M^+); Anal. Calcd (%) for $C_{34}H_{28}Cl_2N_8O_4$: C, 59.74; H, 4.13; N, 16.39. Found C, 59.71; H, 4.21; N, 16.36.

5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-6-(4-methoxyphenyl)-1,3-dimethyl-2,4-dioxo-pyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-6-(4-methoxyphenyl)-1,3-dimethyl-pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4c)

IR (KBr) (ν_{max}/cm^{-1}) 3072.89, 2941.34, 1697.68, 1641.43, 1532.71, 1470.91; δ_H (100 MHz; DMSO-d_6) 3.36 (s, 6H, NCH_3), 3.39 (s, 6H, NCH_3), 3.48 (s, 3H, OCH_3), 6.43 (d, 2H, J = 10.2 Hz, CH), 6.77–7.21 (m, 4H, arom.) 7.68–8.00 (m, 8H, arom.), 8.10 (d, 2H, J = 7.8 Hz, CH=N); δ_C (100 MHz; DMSO-d_6) 28.8, 29.1, 34.8, 58.0, 58.2, 89.7, 123.1, 123.6, 126.9, 127.1, 129.6, 130.1, 132.8, 140.4, 141.3, 142.2, 149.5, 152.7, 153.1, 163.2; MS, m/z 674 (M^+); Anal. Calcd (%) for $C_{36}H_{34}N_8O_6$: C, 64.09; H, 5.08; N, 16.61. Found C, 64.07; H, 5.05; N, 16.65.

5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,4-dioxo-6-*p*-tolylpyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-1,3-dimethyl-6-*p*-tolylpyrimido-[4,5-*d*]pyrimidine-2,4 -(1*H*,3*H*)-dione (4d)

IR (KBr) (ν_{max}/cm^{-1}) 3073.76, 2942.14, 1698.43, 1641.28, 1532.73, 1471.45; δ_H (100 MHz; DMSO-d_6) 2.06 (s, 3H, CH_3), 3.38 (s, 6H, NCH_3), 3.35 (s, 6H, NCH_3), 6.42 (d, 2H, J = 9.87 Hz, CH), 6.75–7.13 (m, 4H, arom.) 7.63–8.00 (m, 8H, arom.), 8.07 (d, 2H, J = 7.24 Hz, CH=N); δ_C (100 MHz; DMSO-d_6) 21.6, 28.9, 29.3, 57.9, 58.1, 89.8, 122.7, 123.1, 126.9, 127.2, 129.6, 129.9, 132.8, 140.3, 141.2, 142.1, 149.1, 152.7, 153.1, 162.5; MS, m/z 642 (M^+); Anal. Calcd (%) for $C_{36}H_{34}N_8O_4$: C, 67.28; H, 5.33; N, 17.43. Found C, 67.31; H, 5.36; N, 17.41.

5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,4-dioxo-6-*o*-tolylpyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-1,3-dimethyl-6-*o*-tolylpyrimido-[4,5-*d*]pyrimidine-2,4 -(1*H*,3*H*)-dione (4e)

IR (KBr) (ν_{max}/cm^{-1}) 3073.76, 2942.14, 1698.43, 1641.28, 1532.73, 1471.45; δ_H (100 MHz; DMSO-d_6) 2.06 (s, 3H, CH_3), 3.31 (s, 6H, NCH_3), 3.34 (s, 6H, NCH_3), 6.39 (d, 2H, J = 10.12 Hz, CH), 6.95–7.20 (m, 4H, arom.) 7.78–8.03 (m, 8H, arom.), 8.07 (d, 2H, J = 8.54 Hz, CH=N); δ_C (100 MHz; DMSO-d_6) 21.5, 28.4, 28.9, 57.6, 58.1, 89.8, 122.8, 123.3, 126.7, 127.2, 129.6, 129.9, 132.8, 139.7, 140.2, 141.2, 142.1, 149.0, 152.5, 154.1, 162.3; MS, m/z 642 (M^+); Anal. Calcd (%) for $C_{36}H_{34}N_8O_4$: C, 67.28; H, 5.33; N, 17.43. Found C, 67.31; H, 5.36; N, 17.41.

5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-6-(4-nitrophenyl)-2,4-dioxo-pyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-1,3-dimethyl-6-(4-nitrophenyl)pyrimido[4,5-*d*]-pyrimidine-2,4(1*H*,3*H*)-dione (4f)

IR (KBr) (ν_{max}/cm^{-1}) 3073.56, 2941.63, 1696.78, 1640.17, 1532.98; δ_H (100 MHz; DMSO-d_6) 3.31 (s, 6H, NCH_3), 3.38 (s, 6H, NCH_3), 5.88 (d, 2H, J = 9.72 Hz, CH), 7.10–7.47 (m, 4H, arom.), 7.63–7.74 (m, 8H, arom.), 8.11 (d, 2H, J = 8.78 Hz, CH=N); δ_C (100 MHz; DMSO-d_6) 30.4, 31.2, 59.1, 59.5, 87.9, 122.6, 123.7, 126.9, 127.2, 129.2, 130.1, 132.3, 140.1, 141.6, 142.7, 148.8, 152.5, 153.1, 162.7; MS, m/z 704 (M^+); Anal. Calcd (%) for $C_{34}H_{28}N_{10}O_8$: C, 57.95; H, 4.01; N, 19.88; Found C, 57.94; H, 4.03; N, 19.87.

5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-6-(2-nitrophenyl)-2,4-dioxo -pyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-1,3-dimethyl-6-(2-nitrophenyl)pyrimido[4,5-*d*]-pyrimidine-2,4(1*H*,3*H*)-dione (4g)

IR (KBr) (ν_{max}/cm^{-1}) 3073.56, 2941.43, 1698.21, 1641.17, 1532.78, 1472.31; δ_H (100 MHz; DMSO-d_6) 3.29 (s, 6H, NCH_3), 3.37 (s, 6H, NCH_3), 5.86 (d, 2H, J = 9.86 Hz, CH), 7.15–7.52 (m, 4H, arom.), 7.70–7.74 (m, 8H, arom.), 8.12 (d, 2H, J = 7.78 Hz, CH=N) ppm; δ_C (100 MHz; DMSO-d_6) 30.4, 33.8, 59.1, 59.4, 87.7, 122.7, 123.7, 126.9, 127.2, 129.1, 130.0, 132.3, 139.7, 140.1, 141.6, 142.7, 148.8, 152.5, 153.1, 162.7; MS, m/z 704 (M^+); Anal. Calcd (%) for $C_{34}H_{28}N_{10}O_8$: C, 57.95; H, 4.01; N, 19.88. Found C, 57.93; H, 4.06; N, 19.87.

5,6-dihydro-5-(4-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-6-(3-nitrophenyl)-2,4-dioxo-pyrimido[4,5-*d*]pyrimidin-5-yl)phenyl)-1,3-dimethyl-6-(3-nitrophenyl)pyrimido[4,5-*d*]-pyrimidine-2,4(1*H*,3*H*)-dione (4h)

IR (KBr) (ν_{max}/cm^{-1}) 3073.56, 2941.63, 1698.31, 1640.17, 1532.78, 1471.41; δ_H (100 MHz; DMSO-d_6) 3.28 (s, 6H, NCH_3), 3.37 (s, 6H, NCH_3), 5.86 (d, 2H, J = 9.4 Hz, CH), 7.10–7.47 (m, 4H, arom.), 7.63–7.74 (m, 8H, arom.), 8.09 (d, 2H, J = 7.78 Hz, CH=N); δ_C (100 MHz; DMSO-d_6) 30.3, 309.8, 59.1, 59.4, 87.9, 122.7, 123.7, 126.9, 127.2, 129.1, 130.0, 132.3, 138.9, 140.1, 141.6, 142.7, 148.8, 152.5, 153.1, 162.7; MS, m/z 704 (M^+); Anal. Calcd (%) for $C_{34}H_{28}N_{10}O_8$: C, 57.95; H, 4.01; N, 19.88. Found C, 57.93; H, 4.06; N, 19.87.

Acknowledgements

We thank S. J. Kalita, P. Bharali and B. K. Konwer for their assistance with modifying the manuscript and bioactivities studies, respectively. This work was supported in part by grants from the DST (Grant number SR/FTP/CS-58/2005), Govt. of India.

Notes and references

1 (*a*) R. A. Hughes, S. P. Thompson, L. Alcaraz and C. J. Moody, *J. Am. Chem. Soc.*, 2005, **127**, 15644–1565; (*b*) O. Koepler, S. Mazzini, M. C. Bellucci, R. Mondelli, A. Baro, S. Laschat, M. Hotfilder, C. Viseur and W. Frey, *Org. Biomol. Chem.*, 2005, **3**, 2848–2858; (*c*) G. W. Goodall and W. Hayes, *Chem. Soc. Rev.*, 2006, **35**, 280–312.

2 (*a*) S. Kobayashi, T. Semba, T. Takahashi, S. Yoshida, K. Dai, T. Otani and T. Saito, *Tetrahedron*, 2009, **65**, 920–933; (*b*) A. de la Hoz, A. Diaz-Ortiz, A. Moreno and F. Langa, *Eur. J. Org. Chem.*, 2000, 3659–3673; (*c*) B. C. Hong, J. Wu, A. Gupta, M. Hallur and J. Liao, *Org. Lett.*, 2004, **6**, 3453–3456.

3 (*a*) H. Oikawa and T. Tokiwano, *Nat. Prod. Rep.*, 2004, **21**, 321–352; (*b*) K. C. Nicolaou, S. A. Snyder, T. Montagnon and G. Vassilikogiannakis, *Angew. Chem., Int. Ed.*, 2002, **41**, 1668–1698; (*c*) K. A. Miller, S. Tsukamoto and R. M. Williams, *Nat. Chem.*, 2009, **1**, 63–68.

4 (*a*) D. L. Boger, Heterodiene Additions, in *Comprehensive Organic Synthesis*, ed. L. A. Paquette, Pergamon, New York, 1991, vol. 5, pp. 451–512; (*b*) M. E. Jung and J. J. Shapiro, *J.*

Am. Chem. Soc., 1980, **102**, 7862–7866; (*c*) C. N. R. Rao, P. R. Basu and M. S. Hedge, *Appl. Spectrosc. Rev.*, 1979, **15**, 1–139; (*d*) K. D. Jordan and P. D. Burrow, *Acc. Chem. Res.*, 1978, **11**, 341–348.

5 M. Behforouza and M. Ahmadian, *Tetrahedron*, 2000, **56**, 5259–5288.

6 E. C. Taylor, R. J. Knof, R. F. Meyer, A. Holmes and M. L. Hoefle, *J. Am. Chem. Soc.*, 1960, **82**, 5711–5718.

7 (*a*) P. Sharma, N. Rane and V. K. Gurram, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 4185–4190; (*b*) P. Sharma, A. Kumar, N. Rane and V. K. Gurram, *Tetrahedron*, 2005, **61**, 4237–4248.

8 K. Saravanan, H. C. Barlow, M. Barton, A. H. Calvert, B. T. Golding, D. R. Newell, J. S. Northen, N. J. Curtin, H. D. Thomas and R. J. Griffin, *J. Med. Chem.*, 2011, **54**, 1847–1859.

9 H.-Z. Yang, M.-Y. Pan, D.-W. Jiang and Y. He, *Org. Biomol. Chem.*, 2011, **9**, 1516–1522.

10 (*a*) D. Prajapati and A. J. Thakur, *Tetrahedron Lett.*, 2005, **46**, 1433–1436; (*b*) D. Prajapati, M. Gohain and A. J. Thakur, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 3537–3540; (*c*) A. J. Thakur, P. Saikia, D. Prajapati and J. S. Sandhu, *Synlett*, 2001, 1299–1301; (*d*) S. Das and A. J. Thakur, *Eur. J. Org. Chem.*, 2011, 2301–2308; (*e*) S. Das, B. K. Saikia, B. Sridhar and A. J. Thakur, *Acta Crystallogr., Sect. E: Struct. Rep. Online*, 2008, **64**, o1662; (*f*) A. J. Thakur, S. Das and A. K. Phukan, *J. Mol. Struct.*, 2009, **929**, 134–140.

11 M. G. Constantino, V. Jr. Lacerda, G. V. J. da Silva, L. Tasic and R. Rittner, *J. Mol. Struct.*, 2001, **597**, 129–136.

12 I. K. Boakye-Yiadom, *Planta Medicia.*, 1979, **54**(2), 126–212.

13 A. Banso and S. O. Adeyemo, *Nig. J. Biotech.*, 2007, **18**, 27–32.

14 P. Radhika, B. S. Sastry and B. H. Madhu, *Res. J. Biotech.*, 2008, **3**(3), 62–63.

15 M. A. Randhawa, *Jpn. J. Med. Mycol.*, 2006, **47**, 313–318.

16 (*a*) A. I. Hussain, F. Anwar, S. A. S. Chatha, A. Jabbar, S. Mahboob and P. S. Nigam, *Braz. J. Microbiol.*, 2010, **41**, 1070–1078; (*b*) S. Prabuseenivasan, M. Jayakumar and S. Ignacimuthu, *BMC Complementary Altern. Med.*, 2006, **6**, 39–46; (*c*) K. Konaté, A. Hilou, J. F. Mavoungou, A. N. Lepengué, A. Souza, N. Barro, J. Y. Datté, B. M'Batchi and O. G. Nacoulma, *Ann. Clin. Microbiol. Antimicrob.*, 2012, **11**, 5–10; (*d*) B. S. Negi and B. P. Dave, *Indian J. Microbiol.*, 2010, **50**(4), 369–374; (*e*) J. A. M. de Paula, M. R. R. do Silva, M. P. Costa, D. G. A. Diniz, F. A. S. S. F. Alves, E. A. Costa, R. C. Lino and J. R. de Paula, *Evidence-Based Complementary Altern. Med.*, 2012, 1–15; (*f*) M. Khurram, M. A. Khan, A. Hameed, N. Abbas, A. Qayum and H. Inayat, *Molecules*, 2009, **14**, 1332–1341.

17 T. Wadhwani, K. Desai, D. Patel, D. Lawani, P. Bahaley, P. Joshi and V. Kothari, *The Internet Journal of Microbiology*, 2009, **7**, DOI: 10.5580/b43.