Res Chem Intermed
DOI 10.1007/s11164-015-2130-2

Synthesis and antimicrobial activity of novel imidazo[1,2-*a*]pyridinopyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones and thioxopyrimidine-4,6(1*H*,5*H*)diones

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Received: 12 March 2015 / Accepted: 3 June 2015

Abstract A novel series of imidazo[1,2-*a*]pyridinopyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones and thioxopyrimidine-4,6(1*H*,5*H*)diones were synthesized via multistep synthesis starting from 2-aminopyridine on cyclisation with phenacyl bromide followed by Vilsmeier–Haack and Knoevenagel condensation reactions. Structures of all the newly synthesized compounds were confirmed by their spectral and analytical studies. All the synthesized compounds were screened for their in vitro antimicrobial activity. Antibacterial activity results revealed that compounds **5f**, **5i,** and **5j** have shown promising activity against *S. pyogenes* with ZOI ranging from 19 to 20 mm, compound **5g** against *P. aeruginosa* (ZOI 19 mm) and **5f** and **5h** against *S. aureus* (ZOI 17, 18 mm) have shown good antibacterial activity. Among barbituric acid and thiobarbituric acid derivatives, thiobarbituric acid derivatives have shown maximum antibacterial activity. None of the compounds were found to be active at 150 μg/mL concentration against tested fungal strains.

Keywords Antimicrobial activity · Barbituric acid · Imidazo[1,2-*a*]pyridine · Thiobarbituric acid

Electronic supplementary material The online version of this article (doi:10.1007/s11164-015-2130-2) contains supplementary material, which is available to authorized users.

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Published online: 16 June 2015

Introduction

Antibiotics have revolutionized medical care in the twentieth century, these are the most important weapons aiding humans against a number of infectious diseases. Bacterial resistance to antibiotics is of grave concern in the medical community as many species of bacteria have evolved resistance to certain antibiotics and synthetic agents. Therefore, there is a rapidly growing global crisis in the clinical management of life-threatening infectious disease caused by multidrug-resistant strains of the Gram-positive pathogens *Streptococcus*, *Staphylococcus,* as well as the Gram-negative pathogens such as *Salmonella*, and certain *Pseudomonas* strains. For example, distribution of methicillin resistant *Staphylococcus aureus* (MRSA) strains has increased [1, 2]. Moreover, the high level of inherent antibiotic resistance in *P. aeruginosa* makes treatment of these infections problematic [3]. Therefore, the development of new, alternative, more effective antimicrobial agents with a broad spectrum of activities is one of the major challenges in drug discovery.

During recent years, there have been intense investigations on imidazopyridine. Literature survey revealed that imidazopyridines are pharmacologically important scaffolds that are known to exhibit a broad spectrum of biological activities like inhibitors of aromatase estrogen production suppressors [4], thromboxane synthetase [5], HCV replication [6], as well as melatonin receptor ligands [7], DNA directed alkylating [8], and cardiotonic agents [9]. This heterocyclic scaffold is also known to exhibit a variety of biological activities such as anticancer [10], anticoccodial [11], anticandidal [12], anticonvulsant [13], anti-inflammatory [14], antimicrobial [15], antioxidant [16], antitubercular [17], hypnoselective, anxioselective [18], and antiviral [19] activities. The scaffold also has a high affinity to human β-amyloid plaques [20]. In addition, imidazopyridines are the major class of non-benzodiazepines, acting upon various central nervous systems (CNS) disorders. Interestingly, several imidazopyridine-based drugs such as zolpidem, alpidem, and saripidem, exhibit potency against pentylenetetrazole (PTZ)-induced seizures [21]. The chemical structure of some important imidazo[1,2-*a*]pyridine-based CNS agents are given in Fig. 1. On the other hand, barbiturate derivatives also possess several biological activities such as anticancer [22], antiviral [23, 24], sedative, hypnotic, antispasmodic, anticonvulsant, and local anesthesia [25], as well as anti-inflammatory [26] activities. Prompted by the above facts and in continuation of our work on barbiturates [27, 28], herein we report the synthesis of novel pyrimidine

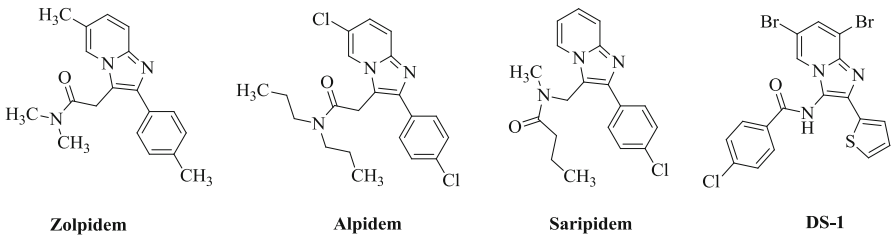


Fig. 1 Imidazo[1,2-*a*]pyridine-based CNS agents

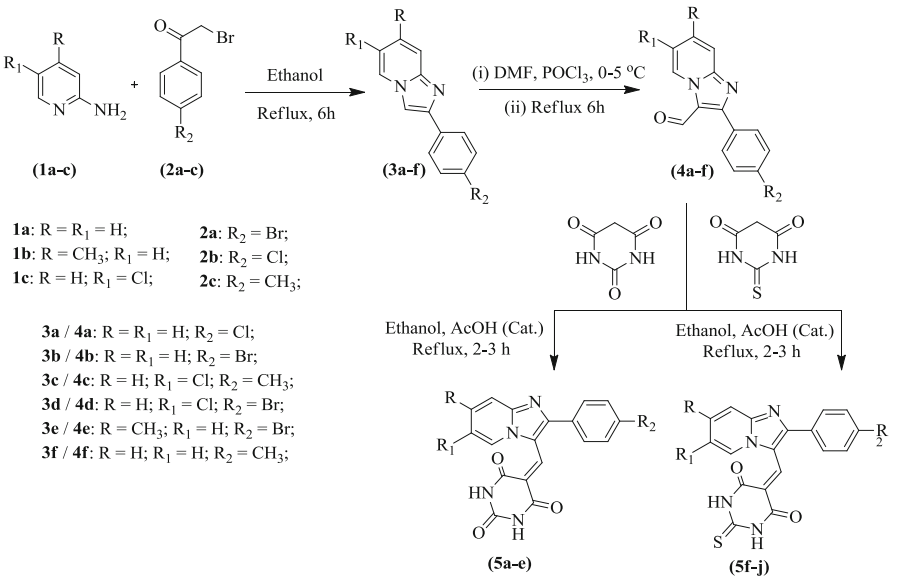
derivatives via Knovenagel condensation reaction between imidazo[1,2-*a*]pyridine-3-carbaldehyde and barbiturates and evaluation of their in vitro antimicrobial activity.

Results and discussion

Chemistry

The target compounds, imidazo[1,2-*a*]pyridinopyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones **(5a–e)** and thioxopyrimidine-4,6(1*H*,5*H*)diones **(5f–j)** were synthesized in good yields, via Knoevenagel condensation of imidazo[1,2-*a*]pyridine-3-carbaldehydes **(4a–f)** with barbituric acid and thiobarbituric acid. The intermediate imidazo[1,2-*a*]pyridine-3-carbaldehydes **(4a–f)** were prepared by cyclization of 2-aminopyridine **(1)** with phenacylbromides **(2a–c)**. The formed imidazo[1,2-*a*]pyridines in Vilsmeier-Haack reaction gave imidazo[1,2-*a*]pyridine-3-carbaldehydes **(4a–f)** [13]. A general approach to synthesize the designed compounds is outlined in Scheme 1.

All the synthesized compounds were characterized by IR, NMR, and mass spectral data. In IR spectra, appearance of a broad band ranging from 3382 to 3475 cm^{-1} attributed to NH group of the barbituric acid and thiobarbituric acid. Bands at 1759–1650 cm^{-1} stretching frequencies correspond to the C=O groups of barbituric acid. In thiobarbiturates C=S stretching frequency was observed in the range of 1246–1292 cm^{-1}. In ^1H NMR spectra absence of aldehyde proton signal at δ 9.78 and presence of a signal in the range of δ 8.19–8.63 ppm (C=C–H) supports



Scheme 1 Synthesis of imidazo[1,2-*a*]pyridinopyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones **(5a–e)** and thioxopyrimidine-4,6(1*H*,5*H*)diones **(5f–j)**

the formation of compounds (**5a–j**). The NH signals of barbituric acid were detected in the 11.20–11.43 ppm range, while the NH signal in thiobarbituric acid was observed in the 11.31–11.64 ppm range. ^{13}C-NMR signal at δ 161.55–163.52 ppm assign the C=O groups in barbituric acid, whereas the signal at 178.01 ppm is attributed to the C=S group in the thiobarbiturc acid derivative. Molecular ion peak from the mass spectrum as well as elemental analyses further confirmed the formation of the product (Table 1).

Biological assay

Antimicrobial activity

In vitro antimicrobial activity of all the newly synthesized compounds was carried out by using agar well diffusion assay [29, 30]. In vitro antibacterial activity was studied against Gram-positive organisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and in vitro antifungal activity against fungal strains such as *Candida albicans*, *Fusarium oxysporum*, *Aspergillus niger,* and *Aspergillus candidus* fungal strains. Antibacterial activity was determined in terms of zone of inhibition (ZOI in mm) at 150 μg/mL concentration and minimum inhibitory concentration (MIC in μg/mL). Antifungal activity was determined in terms of zone of inhibition. Streptomycin for bacteria and clotrimazole for fungi are taken as standard drugs at 30 μg/mL.

In vitro antibacterial activity results (Table 2) revealed that compounds **5f**, **5i**, and **5j** show promising activity against *S. pyogenes* with ZOI ranging from 19 to 20 mm with MIC 50 μg/mL, compound **5g** against *P. aeruginosa* has also shown maximum antibacterial activity with ZOI 19 mm, MIC 50 μg/mL. Compounds **5h**, **5f** against *S. aureus* also have shown good antibacterial activity with ZOI 18, 17 mm, MIC 50 μg/mL. Thus, antibacterial activity results showthat, among barbituric acid and thiobarbituric acid derivatives, thiobarbituric acid derivatives

Table 1 Synthesis of imidazo[1,2-*a*]pyridinopyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones **(5a–e)** and thioxopyrimidine-4,6(1*H*,5*H*)diones **(5f–j)**

Anolog	R	R_1	R_2	Time (h)	Yield[a] (%)
5a	H	H	Cl	2	92
5b	H	H	Br	2.5	88
5c	H	Cl	CH_3	2.5	89
5d	H	Cl	Br	3	86
5e	CH_3	H	Br	3	89
5f	H	H	CH_3	2	94
5g	H	H	Cl	2	92
5h	H	H	Br	2.5	90
5i	H	Cl	Br	3	89
5j	H	Cl	CH_3	2.5	91

Reaction condition: Compounds **4a–f** (1 mmol), barbituric acid/ thiobarbituric acid (1 mmol), EtOH, Cat. AcOH, Reflux 2–3 h

[a] Isolated yields

Table 2 Antibacterial activity of imidazo[1,2-*a*]pyridinopyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones **(5a–e)** and thioxopyrimidine-4,6(1*H*,5*H*)diones **(5f–j)**

S. no	Anolog	*S. aureus*		*S. pyogenes*		*P. aeruginosa*		*K. pneumoniae*	
		ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC
1	**5a**	8	200	10	200	8	200	9	200
2	**5b**	10	200	8	200	8	200	8	200
3	**5c**	8	200	10	200	10	200	10	200
4	**5d**	8	200	10	200	8	200	8	200
5	**5e**	10	200	8	200	10	200	8	200
6	**5f**	17	50	19	50	13	100	12	100
7	**5g**	15	100	13	100	19	50	14	100
8	**5h**	18	50	15	100	14	100	13	100
9	**5i**	14	100	20	50	15	100	16	100
10	**5j**	15	100	19	50	14	100	14	100
11	Streptomycin	22	25	21	12.5	20	12.5	22	50

Zone of inhibition values (mm) for analogs (**5a**–**j**) and positive control drugs (Streptomycin) were measured at 150 and 30 μg/mL, respectively. MIC values were given in μg/mL. Bacterial strains: *S. aureus—Staphylococcus aureus*, *S. pyogenes—Streptococcus pyogenes*, *P. aeruginosa—Pseudomonas aeruginosa* and *K. pneumoniae—Klebsiella pneumonia*

(5f–j) have maximum antibacterial activity. Remaining compounds have shown moderate activity. None of the compounds were found to be active at 150 μg/mL concentration against tested fungal strains.

Conclusion

In conclusion, we have synthesized a novel series of imidazo[1,2-*a*]pyridinopyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones and thioxopyrimidine-4,6(1*H*,5*H*)-diones and evaluated for their antimicrobial activity. Compound **5f, 5i**, and **5j** against *S. pyogenes*, compound **5g** against *P. aeruginosa* and compound **5f**, **5h** against *S. aureus* have shown promising antibacterial activity with ZOI ranging from 17 to 20 mm. Compounds derived from thiobarbituric acid **(5f–j)** have shown better antibacterial activity than the compounds derived from barbituric acid. None of the compounds have shown antifungal activity.

Experimental

Materials and methods

All the reagents were purchased from Aldrich/Merck and used without further purification. Melting points were determined in open capillaries using a Stuart SMP30 apparatus and are uncorrected. The progress of the reactions, as well as

purity of the compounds, was monitored by thin layer chromatography (TLC) with F_{254} silica gel precoated sheets using hexane/ethyl acetate (7/3) as eluent. IR spectra were recorded on a Perkin–Elmer 100S spectrophotometer using KBr pellet. NMR spectra were recorded on Bruker 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Elemental analyses were performed on a Carlo Erba model EA1108, and mass spectra were recorded on a Jeol JMSD-300 spectrometer.

*General procedure for the synthesis of 2-(4-substituted-phenyl)-imidazo[1,2-a]pyridines (**3a–f**)*

4-Substituted phenacylbromide (**2a–c**, 0.1 mol) was added to a solution of substituted 2-amino pyridine (**1a–c**, 0.1 mol) in 15 mL of ethanol, and the reaction mixture was stirred at reflux temperature for 6 h. The progress of the reaction was checked by TLC. After completion of the reaction, the mixture was cooled, and the solid was separated, filtered, and dried in vacuum to obtain analytically pure product in good yields.

*General procedure for the synthesis of 2-(4-substituted-phenyl)-imidazo[1,2-a]pyridin-3-carbaldehydes (**4a–f**)*

Vilsmeyer reagent was prepared at 0–5 °C by dropping phosphorus oxychloride (11 mL), into a stirred solution of DMF (8 mL). Compound (**3a–f**, 3 mmol) was suspended in $CHCl_3$ (15 mL) and dropped into the Vilsmeier reagent, while maintaining stirring and cooling for half an hour. The mixture so obtained was refluxed for 6 h, and the solution was evaporated to dryness in vacuum. The residue was treated with cold water, solid thus obtained was filtered and crystallized from methanol.

*General procedure for the synthesis of imidazo[1,2-a]pyridinopyrimidine-2,4,6(1H,3H,5H)-triones (**5a–e**)*

A mixture of imidazo[1,2-*a*]pyridine-3-carbaldehydes (**4a–e**, 1 mmol) and barbituric acid (1 mmol) were taken in 10 mL of ethanol, and a catalytic amount of acetic acid was added and refluxed for 2–3 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the solid was separated out, filtered, and washed with hot ethanol, which afforded the analytically pure product in good yields.

*5-((2-(4-Chlorophenyl)imidazo[1,2-a]pyridin-3-yl)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (**5a**)* Orange solid, Yield: 90 %; mp: 347 °C; IR (KBr, υ_{max}, cm^{-1}): 3468 (NH), 3184–3033 (CH), 1759, 1697 (C=O), 755 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.19 (t, $J = 6.8$ Hz, 1H), 7.64–7.71 (m, 3H), 7.78 (d, $J = 8.4$ Hz, 2H), 7.88 (d, $J = 8.8$ Hz, 1H), 7.99 (d, $J = 6.8$ Hz, 1H), 8.20 (s, 1H), 11.25 (s, 1H), 11.37 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 113.24, 114.78, 116.52, 118.44, 128.89, 129.65, 131.06, 131.24, 131.82, 134.46, 134.89, 148.04,

150.36, 152.47, 161.55, 163.52; MS (ESI) *m/z*: 367 $[M + H]^+$; Anal. calcd. for $C_{18}H_{11}ClN_4O_3$: C, 58.95; H, 3.02; N, 15.28. Found: C, 58.81; H, 3.23; N, 15.45.

*5-((2-(4-Bromophenyl)imidazo[1,2-a]pyridin-3-yl)methylene)pyrimidine-2,4,6(1H, 3H,5H)-trione (**5b**)* Orange solid, Yield: 87 %; mp: 369 °C; IR (KBr, υ_{max}, cm^{-1}): 3475 (NH), 3181–3032 (CH), 1756, 1697 (C=O), 655 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.19 (t, $J = 6.8$ Hz, 1H), 7.69 (t, $J = 8.4$ Hz, 3H), 7.78 (d, $J = 8.4$ Hz, 2H), 7.88 (d, $J = 8.8$ Hz, 1H), 7.99 (d, $J = 6.8$ Hz, 1H), 8.19 (s, 1H), 11.26 (s, 1H), 11.38 (s, 1H); MS (ESI) *m/z*: 412 $[M + H]^+$; Anal. calcd. for $C_{18}H_{11}BrN_4O_3$: C, 52.57; H, 2.70; N, 13.62. Found: C, 52.39; H, 2.51; N, 13.83.

*5-((6-Chloro-2-(p-tolyl)imidazo[1,2-a]pyridin-3-yl)methylene)pyrimidine-2,4,6(1H, 3H,5H)-trione (**5c**)* Orange solid, Yield: 88 %; mp: 311 °C; IR (KBr, υ_{max}, cm^{-1}): 3460 (NH), 3194–3057 (CH), 1740, 1692 (C=O), 775 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H), 7.39 (d, $J = 8.0$ Hz, 2H), 7.86 (t, $J = 8.4$ Hz, 4H), 8.19 (s, 1H), 8.58 (s, 1H), 11.43 (s, 2H); MS (ESI) *m/z*: 381 $[M + H]^+$ Anal. calcd. for $C_{19}H_{13}ClN_4O_3$: C, 59.93; H, 3.44; N, 14.71. Found: C, 59.77; H, 3.30; N, 14.93.

*5-((2-(4-Bromophenyl)-6-chloroimidazo[1,2-a]pyridin-3-yl)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (**5d**)* Orange solid, Yield: 86 %; mp: 349 °C; IR (KBr, υ_{max}, cm^{-1}): 3383 (NH), 3191–3059 (CH), 1739, 1690 (C=O), 816 (C–Cl), 599 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14 (d, $J = 8.8$ Hz, 2H), 7.68–7.71 (m, 3H), 7.87 (d, $J = 9.2$ Hz, 1H), 8.12 (s, 1H), 8.19 (s, 1H), 11.31 (s, 1H), 11.37 (s, 1H); MS (ESI) *m/z*: 446 $[M + H]^+$ Anal. calcd. for $C_{18}H_{10}BrClN_4O_3$: C, 48.51; H, 2.26; N, 12.57. Found: C, 48.39; H, 2.13; N, 12.75.

*5-((2-(4-Bromophenyl)-7-methylimidazo[1,2-a]pyridin-3-yl)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (**5e**)* Orange solid, Yield: 91 %; mp: 317 °C; IR (KBr, υ_{max}, cm^{-1}): 3473 (NH), 3180–3031 (CH), 1754, 1697 (C=O), 654 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.43 (s, 3H), 7.67 (s, 1H), 7.77 (d, $J = 8.4$ Hz, 2H), 7.87 (t, $J = 8.0$ Hz, 2H), 8.17 (d, $J = 8.8$ Hz, 1H), 8.48 (s, 1H), 8.53 (d, $J = 6.8$ Hz, 1H), 11.20 (s, 1H), 11.33 (s, 1H); MS (ESI) *m/z*: 426 $[M + H]^+$; Anal. calcd. for $C_{19}H_{13}BrN_4O_3$: C, 53.67; H, 3.08; N, 13.18. Found: C, 53.81; H, 3.25; N, 13.03.

*General procedure for the synthesis of imidazo[1,2-a]pyridinothioxopyrimidine-4,6 (1H,5H)diones (**5f–j**)*

A mixture of imidazo[1,2-*a*]pyridine-3-carbaldehydes (**4a–d, 4f**, 1 mmol) and thiobarbituric acid (1 mmol) were taken in 10 mL of ethanol, and a catalytic amount of acetic acid was added and refluxed for 2–3 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the solid was separated out, filtered, and washed with hot ethanol, which afforded the analytically pure product in good yields.

*2-Thioxo-5-((2-(p-tolyl)imidazo[1,2-a]pyridin-3-yl)methylene)dihydropyrimidine-4,6(1H,5H)-dione (**5f**)* Red solid, Yield: 94 %; mp: 339 °C; IR (KBr, υ_{max}, cm^{-1}): 3410 (NH), 3090 (CH), 1650 (C=O), 1292 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H), 7.31 (brs, 1H), 7.38 (d, $J = 8.0$ Hz, 2H), 7.72 (brs, 1H), 7.84 (t, $J = 8.0$ Hz, 2H), 8.20 (d, $J = 7.6$ Hz, 1H), 8.63 (s, 1H), 8.76 (d, $J = 6.8$ Hz, 1H), 11.32 (s, 1H), 11.43 (s, 1H); MS (ESI) *m/z*: 363 $[M + H]^+$; Anal. calcd. for $C_{19}H_{14}N_4O_2S$: C, 62.97; H, 3.89; N, 15.46. Found: C, 62.81; H, 3.73; N, 15.63.

*5-((2-(4-Chlorophenyl)imidazo[1,2-a]pyridin-3-yl)methylene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**5g**)* Red solid, Yield: 92 %; mp: 303 °C; IR (KBr, υ_{max}, cm^{-1}): 3467 (NH), 3114 (CH), 1683, 1651 (C=O), 1286 (C=S), 769 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.25 (t, $J = 6.8$ Hz, 1H), 7.35 (brs, 1H), 7.65 (d, $J = 8.4$ Hz, 2H), 7.75–7.81 (m, 2H), 7.92–8.02 (m, 2H), 8.19 (s, 1H), 11.43 (s, 2H); MS (ESI) *m/z*: 383 $[M + H]^+$; Anal. calcd. for $C_{18}H_{11}ClN_4O_2S$: C, 56.47; H, 2.90; N, 14.64. Found: C, 56.59; H, 2.79; N, 14.50.

*5-((2-(4-Bromophenyl)imidazo[1,2-a]pyridin-3-yl)methylene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**5h**)* Red solid, Yield: 89 %; mp: 315 °C; IR (KBr, υ_{max}, cm^{-1}): 3450 (NH), 3115 (CH), 1686, 1645 (C=O), 1286 (C=S), 527 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.25 (t, $J = 6.8$ Hz, 1H), 7.35 (s, 1H), 7.71–7.80 (m, 3H), 7.92 (t, $J = 8.4$ Hz, 2H), 8.01 (d, $J = 6.8$ Hz, 1H), 8.19 (s, 1H), 11.42 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 113.75, 114.36, 116.47, 119.17, 127.99, 128.72, 131.52, 131.85, 131.95, 132.32, 135.13, 148.48, 153.64, 159.50, 161.86, 178.01; MS (ESI) *m/z*: 428 $[M + H]^+$; Anal. calcd. for $C_{18}H_{11}BrN_4O_2S$: C, 50.60; H, 2.59; N, 13.11. Found: C, 50.48; H, 2.75; N, 13.27.

5-((2-(4-Bromophenyl)-6-chloroimidazo[1,2-a]pyridin-3-yl)methylene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5i) Red solid, Yield: 91 %; mp: 299 °C; IR (KBr, υ_{max}, cm^{-1}): 3382 (NH), 3057 (CH), 1651, 1623 (C=O), 1246 (C=S), 767 (C–Cl), 520 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.13–7.19 (m, 2H), 7.70–7.77 (m, 3H), 7.90 (d, $J = 9.2$ Hz, 1H), 8.12 (s, 1H), 8.23 (s, 1H), 11.42 (s, 1H), 11.64 (s, 1H); MS (ESI) *m/z*: 462 $[M + H]^+$; Anal. calcd. for $C_{18}H_{10}BrClN_4O_2S$: C, 46.82; H, 2.18; N, 12.13. Found: C, 46.66; H, 2.31; N, 12.31.

*5-((6-Chloro-2-(p-tolyl)imidazo[1,2-a]pyridin-3-yl)methylene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**5j**)* Red solid, Yield: 92 %; mp: 312 °C; IR (KBr, υ_{max}, cm^{-1}): 3461 (NH), 3112 (CH), 1650 (C=O), 1286 (C=S), 767 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.41 (s, 3H), 7.39 (d, $J = 7.6$ Hz, 2H), 7.64 (d, $J = 7.6$ Hz, 2H), 7.70 (d, $J = 9.6$ Hz, 1H), 7.88 (d, $J = 9.2$ Hz, 1H), 8.13 (s, 1H), 8.23 (s, 1H), 11.31 (s, 1H), 11.38 (s, 1H); MS (ESI) *m/z*: 397 $[M + H]^+$; Anal. calcd. for $C_{19}H_{13}ClN_4O_2S$: C, 57.50; H, 3.30; N, 14.12. Found: C, 57.32; H, 3.14; N, 14.31.

Antibacterial activity

In vitro antibacterial activity was carried out against four pathogenic Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes)* and Gram-negative bacteria (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) by means of agar well diffusion assay [29, 30]. Streptomycin (30 μg/mL) was used as standard drug. Cell suspension containing 10^8 CFU/mL was prepared and evenly spread on the surface of the agar plates of Mueller–Hinton agar medium using sterile swab sticks. Once the plates had been aseptically dried, 10 mm wells were bored using a sterile cork borer. The samples were first dissolved in dimethyl sulfoxide. The sets of five dilutions (25, 37.5, 75, and 150 μg/mL) of samples were prepared in dimethyl sulfoxide using nutrient agar tubes for calculating MIC values. The samples were tested at a concentration of 150 μg/mL for calculating ZOI. The samples were placed into the wells and the plates were incubated at 37 °C for 24 h for bacterial strains. Antibacterial activity was evaluated by measuring the zone of inhibition (mm) and MIC (the lowest concentration of compounds required to inhibit the growth of the tested microorganisms) values.

Antifungal activity

In vitro antifungal activity was carried out against four pathogenic fungi, *Candida albicans*, *Fusarium oxysporum*, *Aspergillus niger*, and *Aspergillus candidus*, by means of agar well diffusion assay [29, 30]. Clotrimazole was used as standard drug. Cell suspension containing 10^5 spore/mL was prepared and evenly spread on the surface of agar plates of Sabouraud dextrose agar medium using sterile swab sticks. Once the plates had been aseptically dried, 10 mm wells were bored using a sterile cork borer. The samples were first dissolved in dimethyl sulfoxide. The samples (150 μg/mL) were placed into the wells and the plates were incubated at 28 °C for 48 h and the resulting zones of inhibitions were measured.

Acknowledgments The authors are thankful to Siddhardha Busi, Centre for Microbiology, Pondicherry University, for performing antimicrobial activity. The author, RG, is also thankful to the University Grants Commission, New Delhi, for providing senior research fellowship.

Conflict of interest The authors have declared no conflict of interest.

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