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A GREEN SYNTHESIS OF INDOLE DERIVATIVES IN WATER : REACTION OF INDOLE -2,3- DIONES WITH 5,6 DIAMINO-1,3-DIMETHYL-2,4(1*H*,3*H*) PYRIMIDINEDIONE HYDROCHLORIDE AND EVALUATION OF ACTIBACTERIAL ACTIVITY

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ABSTRACT: The reactions of indole-2,3-diones (**1**) with 5,6- diamino-1,3-dimethyl-2,4-(1*H*,3*H*) pyrimidinedione hydrochloride (**2**) have been investigated.Reacting (**1**) and (**2**) in water for 30 minutes gave the hydrazone, 3-[5-(6-amino-1,3-dimethyl -2,4-(1*H*,3*H*) pyrimidinedione)]imino2(1*H*) ones(**3**) only.While reacting **1** and **2** in water for 4-5 hours., the product obtained was 1,3-dimethyl-1*H*-indolo[3,2-g] pteridine-2,4-(3*H*,10*H*) diones (**4**).If tetrabutylammonium bromide (TBAB) used as surfactant under aqueous micellar medium for reaction of **1** and **2** then product obtained were 4',6'-dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-triones (**5**). The structure of the synthesized compounds were characterized by their spectral (IR, ^{1}H-NMR, ^{13}C-NMR, Mass) and analytical data.The synthesized compounds were evaluated for antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* bacteria.

KEYWORDS:3-[5-(6-amino-1,3-dimethyl -2,4-(1*H*,3*H*) pyrimidinedione)]imino2(1*H*) ones ;1,3-dimethyl-1*H*-indolo[3,2-g] pteridine-2,4-(3*H*,10*H*) diones; 4',6'-dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-triones, antibacterial activity.

INTRODUCTION:

Heterocyclic scaffolds represent the central framework of many biological active compounds.Among various heterocyclic system,the indole ring systemhas become an important structural component in many pharmaceutical agents.Some of its derivatives show wide range of antibacterial,antifungal,anti-inflammatory, anticancer,anti-HIVactivities.[i,ii]Indole-2,3-diones(isatin)showantimicrobial,[iii]antioxident,[iv] CNS depressant[v]etc.activities.Pyrimidine derivatives are potential anticancer agents.[vi]

The general interest in pteridines is due to their widespread occurrence in both animal and plant kingdom implying potential activity and drug type properties in structural analogues.[vii-xi]Imidazole derivatives show extensive spectrum of biological activities[xii] such as antibacterial,anticancer,antitubercular,antifungal, analgesic and anti-HIV activities.Itwas assumed that combining effect of indolo-pyrimidinedione[xiii,xiiv],indolo-pteridines and indolo-

imidazo-pyrimidine might results in the formation of some novel molecules with enhanced biological activities.

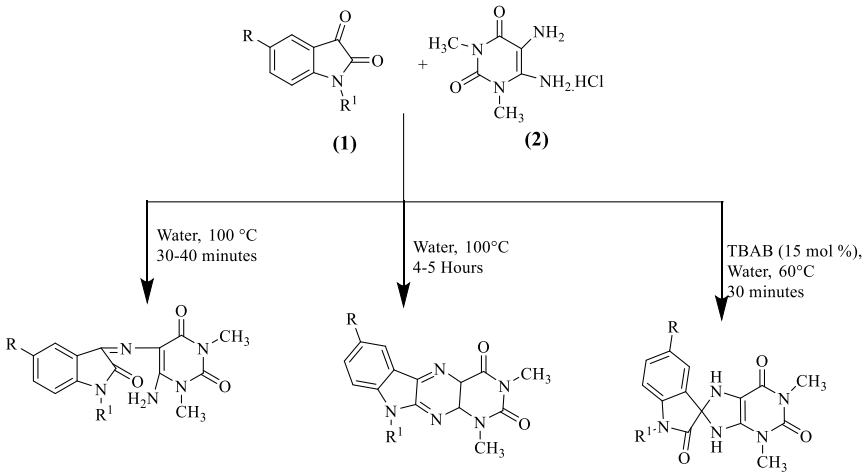
We herein, reporting the reaction of indole-2,3-diones (**1**) with 5,6- diamino-1,3-dimethyl-2,4-(1*H*,3*H*) pyrimidinedione hydrochloride (**2**) for the first time.Currently challenges to built up innovative organic transformations that are not only efficient selective and high yielding but also environmentally benign make the choice of suitable reaction medium necessary for triumphant synthesis.

Exploitation of heterocycles should allow the synthetic chemists to rapidly discover methodology for preparation of complex molecules in shorter reaction time.Among alternatives water as a solvent for organic transformations offer green chemistry benefits.[xv]

In continuation of our work on indole-2,3-diones[xv-xxi]with diamines,[xvii]we investigated the reaction of indole-2,3-dione (**1**) with 5,6-diamino-1,3-dimethyl-2-(1*H*,3*H*) pyrimidinedione hydrochloride (**2**) in aqueous medium.Reaction of **1** and **2** for 30 minutes gave hydrazone 3-[5-(6-amino-1,3-dimethyl -2,4-(1*H*,3*H*) pyrimidinedione)]imino2(1*H*) ones(**3**).When reacting **1** and **2** for 4-5 hours, 1,3-dimethyl-1*H*-indolo[3,2-g] pteridine-2,4-(3*H*,10*H*) diones (**4**) were obtained.

Recently, surfactants have attracted considerable interest in organic synthesis because of their high catalytic activity as well as benign character.[xxii]The use of surfactants in aqueous medium increases reactivity via the formation of micelles.[xxii,xxiii]

Reacting **1** and **2** using tetrabutyl ammonium bromide (TBAB) as a surfactants under aqueous micellar medium gave 4',6'-dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-triones (**5**).(**Scheme-1**).



(1) **(2)**

(3 a-c) **(4 a-d)** **(5 a-f)**

Compound	R	R^1	Compound	R	R^1
3a	H	H	**5a**	H	H
3b	F	H	**5b**	F	H
3c	H	CH_3	**5c**	Cl	H
4a	H	H	**5d**	H	CH_3
4b	F	H	5e	H	CH_2CH_3
4c	Cl	H	5f	H	CH_2Ph
4d	H	CH_3			

Scheme-1. Synthesis of compounds **3a-c**, **4a-d** and **5a-f**.

EXPERIMENTAL :

Chemistry

Melting points are uncorrected and were taken in open glass capillaries using a Gallenkamp melting point apparatus. The IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer in KBr pellets and band positions are recorded in wave numbers (cm^{-1}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ with TMS as an internal reference on a JEOL spectrometer at 300 and 75 MHz, respectively. The mass spectra were recorded on XEVO G2S QTOF-YDA220 mass spectrometer and FAB mass spectra on GEOL SX102/Da-600 mass spectrometer. The elemental analyses were performed at Central Drug Research Institute, Lucknow, India. Chemicals were purchased from *Acros Organics*. 5,6- Diamino-1,3-dimethyl-2,4-(1*H*,3*H*) pyrimidinedione hydrochloride were prepared using standard method.[xxiv-xxvi] The bacterial cultures *Bacillus subtilis*(ATCC 6633), *Staphylococcus aureus*(MTCC 740), *Escherichia coli*(ATCC 25922),and *Pseudomonas aeruginosa* (ATCC 25668) were procured from microbial type culture collection, Institute of Microbial Technology, Chandigarh, India. Dimethyl sulfoxide (DMSO) and Chloramphenicol (standard broad spectrum antibiotics) were used as controls.

General procedure for synthesis of 3-[5-(6-amino-1,3-dimethyl -2,4-(1*H*,3*H*) pyrimidinedione)]imino2(1*H*) ones(3a-c)

A solution of 5,6- diamino-1,3-dimethyl-2,4-(1*H*,3*H*) pyrimidinedione hydrochloride(**2**,0.01mol) in distilled water (50.0 mL) was neutralized with ammonia to PH=7,then indole-2,3-dione (**1**,0.012mol) was added and heated under reflux for 30 minutes.After completion of the reaction the orange solid obtained was collected by filtration and washed with warm water.The crude product so obtained was purified by crystallization from ethanol to afford pure products (**3a-c**).

3-[5-(6-Amino-1,3-dimethyl -2,4-(1*H*,3*H*) pyrimidinedione)]imino2(1*H*) one(3a)

Yield:91%; M.P. : 354-356°C; IR (KBr, υ_{max} , cm^{-1}):3380,3250(NH_2),3190(NH), 1725(>C=O of indole) ,1710 (CO),1690(CO),1620(C=N);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :3.68 (s, 3H, NCH_3), 3.84 (s, 3H, NCH_3),4.98(s,2H,NH_2), 6.40–7.85 (m, 4H,Ar-H), 9.25 (s, 1H,NH); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) :35.42(NCH_3), 37.56(NCH_3), 116.50, 118.32, 120.67, 122.33, 126.08, 129.33, 136.89, 142.80(Ar-C), 162.40(C=N),186.60 (CO), 188.82(CO),189.02(CO).MS. Calcd. for$C_{14}H_{13}N_5O_3$: 299.1018Found: 299.1020. Anal. Calcd for$C_{14}H_{13}N_5O_3$: C, 56.18; H, 4.39; N, 23.40%.Found: C, 56.22; H, 4.40; N, 23.42%.

5-Fluoro 3-[5-(6-amino-1,3-dimethyl -2,4-(1*H*,3*H*) pyrimidinedione)]imino2(1*H*) one(3b)

Yield: 92 %; M.P. : 346-348°C; IR (KBr, υ_{max} , cm^{-1}):3370,3240(NH_2),3192(NH), 1730(>C=O of indole) ,1710 (CO),1685(CO),1620(C=N);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :3.64 (s, 3H, NCH_3), 3.86 (s, 3H, NCH_3),4.96(s,2H,NH_2), 6.50–7.86 (m, 4H,Ar-H), 9.10 (s, 1H, NH); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) :35.82(NCH_3), 37.46(NCH_3), 116.80, 118.42, 120.69, 122.43, 126.28, 129.40, 136.92, 142.40 (Ar-C), 162.62(C=N),186.50(CO),188.50 (CO),189.04 (CO).MS.Calcd.for $C_{14}H_{12}FN_5O_3$: 317.0924Found: 317.0926. Anal. Calcd for $C_{14}H_{12}FN_5O_3$: C, 53.00; H, 3.81; N, 22.07%. Found: C, 53.04; H, 3.85; N, 22.10%.

1-Methyl 3-[5-(6-amino-1,3-dimethyl -2,4-(1*H*,3*H*) pyrimidinedione)]imino2(1*H*) one(3c)

Yield: 90 %; M.P. : 333-335°C; IR (KBr, υ_{max} , cm^{-1}):3375,3250(NH_2), 1730(>C=O of indole) ,1710 (CO),1695(CO),1620(C=N);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15MHz):3.25(s, 3H, NCH_3), 3.69 (s, 3H, NCH_3), 3.85 (s, 3H, NCH_3),4.98(s,2H,NH_2), 6.38–7.84 (m, 4H,Ar-H) ;

^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) :34.51(NCH_3), 35.72(NCH_3), 37.56(NCH_3), 116.40, 118.62, 120.82, 122.65, 126.25, 129.23, 136.90, 142.75 (Ar-C), 162.36(C=N),186.55(CO),188.86(CO), 189.05 (CO).MS. Calcd. for $C_{15}H_{15}N_5O_3$:313.3170 Found: 313.3175. Anal. Calcd for $C_{15}H_{15}N_5O_3$: C, 57.50; H, 4.83; N, 22.35%. Found: C, 57.53; H, 4.86; N, 22.38%.

General procedure for synthesis of 1,3-dimethyl-1*H*-indolo[3,2-g] pteridine-2,4-(3*H*,10*H*) diones(4a-d)

A solution of 5,6- diamino-1,3-dimethyl-(1*H*,3*H*) pyrimidinedione hydrochloride(**2**,0.01mol) in distilled water (50.0 mL) was neutralized with ammonia to PH=7 ,then indole-2,3-dione (**1**,0.012mol) was added and heated under reflux for 5-6 hours. After completion of the reaction the yellowish-white solid obtained was collected by filtration and washed with warm water.The crude product so obtained was purified by crystallization from ethanol to give pure compounds (**4a-d**).

1,3-Dimethyl-1*H*-indolo[3,2-g] pteridine-2,4-(3*H*,10*H*) dione (4a)

Yield: 92 %; M.P. : 296-298˚C; IR (KBr, υ_{max} , cm^{-1}):3195(NH), 1715 (CO),1680(CO),1630(C=N),1620(C=N);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :3.60 (s, 3H, NCH_3), 3.79 (s, 3H, NCH_3), 6.25–7.90 (m, 4H,Ar-H), 9.28 (s, 1H, NH); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6,75.47 MHz) :34.82(NCH_3), 37.65(NCH_3), 116.85, 118.52, 121.07,122.58,126.08,130.33,142.89,148.86(ArC),161.86(C=N),162.30(C=N),186.78(CO),188.06(CO).MS. Calcd. for $C_{14}H_{13}N_5O_2$:283.2910 Found: 283.2914. Anal. Calcd for $C_{14}H_{13}N_5O_2$: C, 59.36; H, 4.63; N, 24.72%. Found: C, 59.39; H, 4.65; N, 24.76%.

7-Fluoro-1,3-dimethyl-1*H*-indolo[3,2-g] pteridine-2,4-(3*H*,10*H*) dione (4b)

Yield: 93 %; M.P. : 286-288˚C IR (KBr, υ_{max}, cm^{-1}):3190(NH), 1710 (CO),1690(CO),1630(C=N),1620(C=N);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :3.63 (s, 3H, NCH_3), 3.82 (s, 3H, NCH_3), 6.28–7.95 (m, 3H,Ar-H), 9.25 (s, 1H, NH); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) :34.80(NCH_3), 37.50(NCH_3), 116.81, 118.62, 121.37, 122.80, 126.28, 130.43, 142.92, 148.65 (Ar-C), 161.90(C=N), 162.35(C=N), 186.70(CO),188.10(CO).MS. Calcd. for $C_{14}H_{12}FN_5O_2$: 301.2814 Found: 301.2817. Anal. Calcd for $C_{14}H_{12}FN_5O_2$: C, 55.81; H, 4.01; N, 23.25%. Found: C, 55.85; H, 4.05; N, 23.28%.

7- Chloro-1,3-Dimethyl-1*H*-indolo[3,2-g] pteridine-2,4-(3*H*,10*H*) dione (4c)

Yield: 91 %; M.P. : 290-292˚C; IR (KBr, υ_{max}, cm^{-1}): 3190(NH), 1705 (CO),1690(CO),1630(C=N),1620(C=N);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :3.65 (s, 3H, NCH_3), 3.84 (s, 3H, NCH_3), 6.27–7.86 (m, 3H,Ar-H), 9.18 (s, 1H, NH); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) :34.82(NCH_3), 37.61(NCH_3), 116.50, 118.59,121.40,122.70,126.50,130.46,142.87,148.70(ArC),162.30(C=N),161.86(C=N),186.75 (CO),188.18(CO).MS. Calcd. for $C_{14}H_{12}ClN_5O_2$:317.7330 Found: 317.7334. Anal. Calcd for $C_{14}H_{12}ClN_5O_2$: C, 52.92; H, 3.81; N, 22.04%. Found: C, 52.94; H, 3.84; N, 22.09%.

1,3,10-Trimethyl-1*H*-indolo[3,2-g] pteridine-2,4-(3*H*,10*H*) dione (4d)

Yield: 91 %; M.P. : 277-279˚C; IR (KBr, υ_{max}, cm^{-1}): 1708 (CO),1695(CO),1630(C=N),1620(C=N);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :3.28 (s, 3H, NCH_3), 3.64 (s, 3H, NCH_3), 3.81 (s, 3H, NCH_3), 6.26–7.86 (m, 4H,Ar-H); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) :3450(NCH_3), 35.79(NCH_3), 37.61(NCH_3), 116.80, 118.52, 121.07, 122.58,126.08, 130.33, 142.89, 148.68 (Ar-C), 161.90(C=N), 162.32(C=N),

186.72(CO),188.10(CO).MS. Calcd. for $C_{15}H_{15}N_5O_2$:297.3180 Found: 297.3183. Anal. Calcd for $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.56%. Found: C, 60.63; H, 5.11; N, 23.59%.

General procedure for synthesis of 4',6'-dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-triones (5a-f)

To a solution of 5,6-diamino-1,3-dimethyl-(1*H,3H*) pyrimidinedione hydrochloride(**2**,0.01mol) in distilled water (50.0 mL) was neutralized with ammonia to PH=7, was added indole-2,3-dione (**1**,0.01mol) and TBAB (0.0483g,15mol %).This reaction mixture was allowed to stir magnetically at 60^0C.Progress of the reaction was monitored by TLC (Pet.ether:ethyl acetate=4:1) and visualization was accomplished in iodine chamber/UV lamp.After completion of the reaction, the solid obtained was collected by filtration and washed with warm water.The crude product so obtained was purified by crystallization with ethanol to give pure compounds **5a-f**.

4',6'-Dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-trione (5a)

Yield: 95 %; M.P. : 238-240 ˚C; IR (KBr, υ_{max}, cm^{-1}): 3195(NH),3185(NH),3180(NH), 1725 (CO),1708(CO),1680(CO);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) : 3.65 (s, 3H,NCH_3),3.87(s,3H,NCH_3),6.287(m,4H,ArH),8.05(s,1H,NH),8.56(s,1H,NH),9.15(s,1H,NH) ;^{13}CNMR(δ,$CDCl_3$+DMSOd_6,75.47MHz):35.65(NCH_3),37.72(NCH_3),108.51(spiro-C),110.90,116.52,122.60,126.18,128.26,130.35,142.80,145.80(ArC),186.95(CO),188.70(CO) ,189.09(CO). MS. Calcd. for $C_{14}H_{13}N_5O_3$:299.2900 Found:299.2904. Anal. Calcd for $C_{14}H_{13}N_5O_3$: C, 56.18; H, 4.38; N, 23.40%. Found: C, 56.22; H, 4.41; N, 23.43%.

5-Fluoro-4',6'-Dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-trione (5b)

Yield: 95 %; M.P. : 228-230 ˚C; IR (KBr, υ_{max}, cm^{-1}):3196(NH), 3187(NH), 3182(NH), 1730 (CO),1710(CO),1685(CO);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) : 3.64 (s, 3H,NCH_3),3.85(s,3H,NCH_3),6.257.15(m,3H,ArH),8.08(s,1H,NH),8.51(s,1H,NH),9.09(s,1H, NH);^{13}CNMR(δ,$CDCl_3$+DMSO-d_6,75.47MHz):35.60(NCH_3),37.69(NCH_3),108.46(spiro-C), 110.50, 116.52, 122.58, 126.08,128.26, 130.33, 142.89, 145.92 (Ar-C), 186.95(CO),188.70(CO),189.09(CO). MS. Calcd. for $C_{14}H_{12}FN_5O_3$: 317.2804 Found: 317.2809. Anal. Calcd for $C_{14}H_{12}FN_5O_3$: C, 53.01.; H, 3.81; N, 22.07%. Found: C, 53.05; H, 3.85; N, 22.12%.

5-Chloro-4',6'-Dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-trione (5c)

Yield: 93 %; M.P. : 234-236˚C; IR (KBr, υ_{max}, cm^{-1}): 3194(NH), 3186(NH), 3181(NH), 1725(CO),1708(CO),1680(CO);^{1}HNMR(δ,$CDCl_3$+DMSOd_6,300.15MHz):3.62(s,3H,NCH_3), 3.81(s,3H,NCH_3),6.207(m,3H,ArH),8.05(s,1H,NH),8.45(s,1H,NH),9.12(s,1H,NH);^{13}CNMR(δ,$CDCl_3$+DMSO-d_6,75.47MHz):35.62(NCH_3),37.56(NCH_3),108.50(spiro-C), 110.62, 116.65, 122.38, 126.18,128.30, 130.45, 142.78, 145.85 (Ar-C), 186.83(CO),188.65(CO),189.05(CO). MS. Calcd. for$C_{14}H_{12}ClN_5O_3$: 333.7320 Found: 333.7324. Anal. Calcd for $C_{14}H_{12}ClN_5O_3$: C, 50.39; H, 3.62; N, 20.99%. Found: C, 50.41; H, 3.65; N, 21.01%.

1,4',6'-Trimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-trione (5d)

Yield: 94 %; M.P. : 236-238 ˚C; IR (KBr, υ_{max}, cm^{-1}): 3185(NH), 3180(NH), 1725 (CO),1705(CO),1680(CO);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :3.25(s, 3H,NCH_3),3.68(s,3H,NCH_3),3.86(s,3H,NCH_3),6.267.12(m,4H,ArH),8.12(s,1H,NH),8.39(s,1

H,NH);^{13}CNMR(δ,$CDCl_3$+DMSOd_6,75.47MHz):34.45(NCH_3),35.68(NCH_3),37.45(NCH_3),108.08(spiro-C),110.65,116.52,122.60,126.18,128.26,130.35,142.80,145.90(Ar-C),186.22(CO),188.58(CO),189.06(CO). MS. Calcd. For$C_{15}H_{15}N_5O_3$: 313.3170 Found: 313.3175. Anal. Calcd for $C_{15}H_{15}N_5O_3$: C, 57.50; H, 4.83; N, 22.35%. Found: C, 57.53; H, 4.87; N, 22.39%.

1-Ethyl-4',6'-Dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5',7'-trione (5e)

Yield: 93 %; M.P. : 242-244 ˚C; IR (KBr, υ_{max}, cm^{-1}):3186(NH), 3181(NH),1725 (CO) ,1715 (CO),1695(CO);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15MHz): 1.85(t,J=7.25Hz,3H, NCH_2**CH_3**), 3.62 (s, 3H, NCH_3), 3.85 (s, 3H, NCH_3),4.03(q,J=7.25Hz,2H,N**CH_2**CH_3), 6.28–7.15 (m, 4H,Ar-H),8.16(s,1H,NH),8.35(s,1H,NH) ; ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 28.82(NCH_2**CH_3**),35.71(NCH_3), 37.62(NCH_3),48.41(N**CH_2**CH_3), 108.26(spiro-C),110.82,116.78,122.87,126.28,128.30,130.39,142.86,145.86(ArC),186.23(CO),188.52(CO) ,189.82(CO). MS. Calcd. for $C_{16}H_{17}N_5O_3$:327.3440 Found: 327.3444. Anal. Calcd for $C_{16}H_{17}N_5O_3$: C, 58.71; H, 5.23; N, 21.39%. Found: C, 58.74; H, 5.26; N, 21.42%.

1-Benzyl-4',6'-dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5',7'-trione (5f)

Yield: 90 %; M.P. : 233-235˚C; IR (KBr, υ_{max}, cm^{-1}):3185(NH), 3180(NH),1730 (CO) ,1708(CO),1685(CO);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15MHz): 3.65 (s, 3H, NCH_3), 3.87 (s,3H,NCH_3),4.08(s,2H,N**CH_2**C_6H_5),6.25–7.85 (m, 9H,Ar-H) ,8.17(s,1H,NH),8.42(s,1H,NH); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) :35.72(NCH_3),37.52(NCH_3),48.62(N**CH_2**C_6H_5), 108.12(spiroC),110.82,112.46,116.78,118.34,120.78,122.87,123.56,126.28,128.30,130.39,132.23,135.12,142.86,145.85(Ar-C),186.43(CO),188.49(CO),189.06(CO). MS. Calcd. for $C_{21}H_{19}N_5O_3$:389.4150 Found: 389.4154. Anal. Calcd for $C_{21}H_{19}N_5O_3$: C, 64.77; H, 4.93; N, 17.98%. Found: C, 64.80; H, 4.97; N, 17.96%.

ANTIBACTERIAL ACTIVITIES :

For antibacterial assay the zone of inhibition was performed at 128µgmL^{-1} concentration for all the compounds (**3a-c,4a-d and5a-f**) using discs diffusion method,[xxvii,xxviii]for this purpose DMSO was used as diluents, Mueller-Hilton (Himedia, India) agar medium was prepared and sterilized by autoclaving at 121˚c at 15 psi for 15 minutes. The medium was poured into sterile petri dishes under aseptic condition using laminar airflow chamber. After the solidification of medium the suspension of the test organism (10^6 cfu mL^{-1}) was swabbed onto the individual media plates using a sterile glass spreader. A sterile discs (9.0mm diameter) impregnated with compounds was placed over media surface and the plates were incubated at 37˚c for 18-24 hours under dark condition. The determination as to whether the organism is susceptible, intermediate or resistant was made by measuring the zone of inhibition in comparison with standard antibiotic.

MIC assay was performed to determine the lowest concentration of compound necessary to inhibit a test organism. MIC values were evaluated for all the compounds **(5a-g)** using broth micro-dilution method as per the standard guideline.[xxvix]Assay was carried out for the compounds at 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 and 128.0 µg mL^{-1} concentrations. A set of tubes containing Muller Hilton broth medium with different concentrations of compounds were prepared. The tubes were inoculated with bacterial culture (10^6 cfu mL^{-1}) and incubated on a rotator shaker (180 rpm) at 37˚c for 18-24hours under dark condition. MIC values were defined as lowest concentration of compound that prevented the visible growth of bacteria after the incubation period. All the experiment were performed in there replicates,

Results and discussion

The antibacterial screening data revealed that all the tested compounds (**3a-c,4a-d and5a-f**) showed moderate to significant bacterial inhibition (**Table 1**). Compounds **3a**, **3b**, **4b,4c,5a,5b** and **5c** were even more potent than the standard antibiotic (Chloramphenicol) against *B. subtilis, S. aureus, E. coli, P. aeruginosa* bacteria. Compound **4a** showed significant bacterial inhibition with *P. aeruginosa* even then standard and equally potent with *B. subtilis*, *S. aureus*and*E. coli*.

Compounds **3b,4b** and **5b** shows equal MIC µg mL^{-1} (**Table 2**) values than standardChloramphenicol.Other compounds show good to moderate zone of inhibition and MIC values.Among all the compounds **3b,4b** and **5b** shows good results due to fluoro substitution in the ring and can be used a potential broad spectrum antibacterial agents as they are potent against both gram positive and gram negative bacteria.

Table-1:**Antibacterial activity 3a-c,4a-d and5a-f.**

Compound	***B.subtilis***	***S.aureus***	***E. coli***	***P.aeruginusa***
	Zone of Inhibition (mm)			
3a	28	24	23	24
3b	32	29	26	30
3c	23	20	20	19
4a	26	24	23	28
4b	32	29	26	28
4c	30	28	25	27
4d	24	20	21	20
5a	27	25	23	25
5b	32	29	26	30
5c	31	28	25	28
5d	24	20	20	22
5e	22	19	18	20
5f	21	18	17	19
Chloramphenicol	26	24	23	25

Table-2 :MIC (µg mL^{-1}) values3a-c,4a-d and5a-f.

Compound	***B.subtilis***	***S.aureus***	***E. coli***	***P.aeruginusa***
	MIC (µgmL^{-1})			
3a	32	32	32	32
3b	16	16	16	16
3c	64	64	64	64
4a	32	32	32	32
4b	16	16	16	16
4c	32	32	32	32
4d	64	64	64	64
5a	32	32	32	32
5b	16	16	16	16
5c	32	32	32	32
5d	64	64	64	64

5e	128	128	128	128
5f	128	128	128	128
Chloramphenicol	16	16	16	16

RESULTS AND DISCUSSION:

Chemistry

The present investigation describes the synthesis of some new functionalized 3-[5-(6-amino-1,3-dimethyl -2,4-(1*H*,3*H*) pyrimidinedione)]imino2(1*H*) ones (**3a-c**); 1,3-dimethyl-1*H*-indolo[3,2-g] pteridine-2,4-(3*H*,10*H*) diones (**4a-d**);4',6'-dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-triones (**5a-f**) by the reaction of 5,6- diamino-1,3-dimethyl-(1*H*,3*H*) pyrimidinedione hydrochloride (**2**) in distilled water with indole-2,3-diones (**Scheme 1**).The formation of product depends on the site of condensation,time of reaction and presence /absence of surfactant.

The reaction of (**1**) and (**2**) in aqueous medium involves the intermediate formation of Schiff's base (**3a-c**).NH_2 group at position 5 of compound (**2**) is expected to be more basic attacking at the β-carbon atom of isatin(**1**) moiety.Formation of Schiff's base was indicated by the appearance of red orange colour of the reaction mixture immediately on mixing the reactant.It is formed within 30 minutes of refluxing **1** and **2** in water as indicatedbyTLC.On increasing the refluxing time 4-5 hours, colour intensity of the reaction mixture decreased and TLC studies indicated the conversion of Schiff's base into cyclized product indolo[3,2-g]pteridine (**4a-d**).

Further, reaction of **1** and **2** was also carried out using surfactant,TBAB(tetrabutyl ammonium bromide) in aqueous medium at ± 60^0C until completion of the reaction evidenced by TLC.We have carried out the reaction at different temperature and results are presented in **Table 3**.It is clear that rate of reaction increases with increasing the catalytic concentration upto 15 mole % without any difference on further increasing catalyst.It is noticed that amount of catalyst plays a significant role in controlling the rate of reaction.Therefore,among various amounts of catalyst studied 15 mole % of TBAB was found to be best at 60 ^{0}C temperature in aqueous medium to give the spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine] (**5a-f**).

It was observed that use of TBAB followed by stirring, the initially floating reactants in the mixture converted to a yellowish-brown turbid emulsion (**Fig. 1a**), which implies the formation of micelle like colloidal aggregates. The light microscopic observation of the emulsion dispersion formed from indole-2,3-dione **1a**, 5,6- diamino-1,3-dimethyl-(1*H*,3*H*) pyrimidinedione hydrochloride (previously neutralized by ammonia solution)**2**and TBAB in distilled water shows that spherical particles were formed (**Fig. 1b**). It is assumed that most of the organic substrates are concentrating in the spherical particles which act as a hydrophobic reaction site and result in rapid reaction in water. Reactant **1a** and 5,6- diamino-1,3-dimethyl-2,4-(1*H*,3*H*) pyrimidinedione hydrochloride (**2**) are hydrophobic in aqueous medium. In the micellar solution these escape from water molecule towards the hydrophobic core of the micelle droplets where reaction occurred. The hydrophobic interior of the micelles rapidly excludes the water molecules produced during the reaction and shifts the equilibrium towards the product side. This is shown schematically in **Figure 1a, b.**

Table 3:**Investigation for the synthesis of 4a under different reaction conditions.[a]**

S. No.	Solvent	Temperature (˚C)	Reaction Time (h)	Yield[b](%)
1.	Water	Reflux	3	73
2.	Water /TBAB[c]	RT	6	85
3.	Water /TBAB[c]	60	0.5	85
4.	Water/TBAB[c]	80	0.5	82

5.	Water /TBAB[c]	100	0.5	81
9.	Water /TBAB[c]	Reflux	0.5	78
6.	Water /TBAB[d]	60	0.5	82
7.	Water /TBAB[e]	60	0.5	90
8.	Water /TBAB[f]	60	0.5	91

[a] Reaction conditions: 0.01 mole of **1a** and 5,6- diamino-1,3-dimethyl-2,4-dioxopyrimidine hydrochloride (**2**) was used.
[b] Isolated yield.
[c] TBAB (10 mol %)
[d]TBAB (5 mol %)
[e]TBAB (15 mol %)
[f]TBAB (20 mol %)

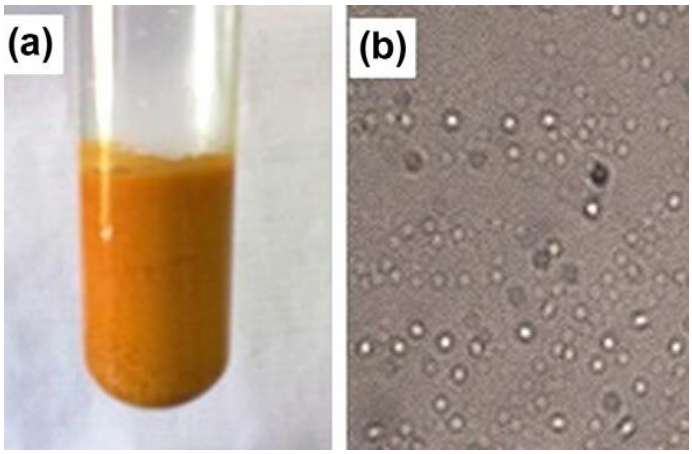


Figure 1. (**a**) Reaction mixture of indole-2,3-dione**1a**and 5,6- diamino-1,3-dimethyl-2,4-(1*H*,3*H*) pyrimidinedione hydrochloride (**2**)and TBAB in distilled water.
(**b**) Optical micrograph of the reaction mixture (scale bar = 25 µm)

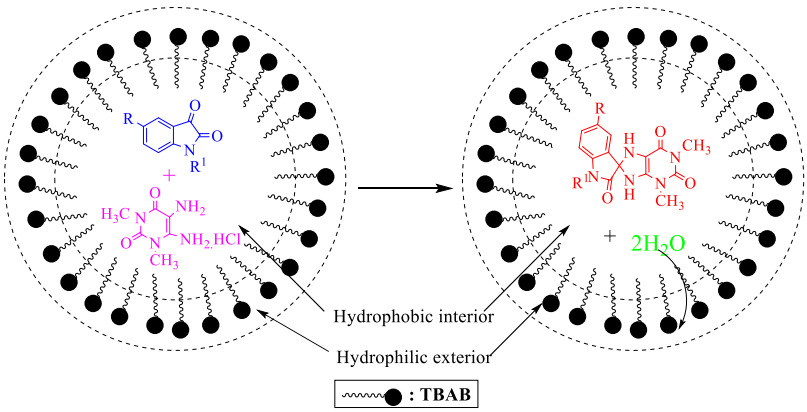


Figure2. Micelles-promoted green synthesis of 4',6'-dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-triones (**5a-f**).

Formation of compounds (**3**) were confirmed on the basis of IR spectra,**3a** shows characteristic absorption at 3380,3250(NH_2),3190(NH),1725(CO,indolyl),1710(CO),1690(CO) and1620(C=N) cm^{-1} . ^{1}H-NMR spectra shows peaks at δ 3.68(NCH_3), 3.84(NCH_3),4.98(NH_2),6.40-7.85(aromatic protons) and 9.25ppm due to NH of indolyl ring. ^{13}C-NMR signal appeared atδ 35.42(NCH_3), 37.56(NCH_3),116.50-142.80(aromatic

carbons),162.40(C=N),186.60(CO) and 189.02(CO) ppm due to indolyl ring.Further,in the mass spectrum molecular ion peak appeared at (m/z) 299.1018 (M^+).

The structure of indolo[3,2-g]pteridines (**4**) were confirmed by IR spectra,which shows complete disappearance of absorption band due to >CO of indole-2,3-diones (**1**) and NH_2 group of pyrimidine moiety(**2**) and appearance of peak due to C=N group in ring system of (4) at1630 and 1620 cm^{-1}.^{1}H-NMR spectra shows disappearance of peak due to NH_2 group of pyrimidine ring of compound **2**. ^{13}C-NMR spectra shows characteristic peak due to C=N at 162.30 and161.86 ppm(**4a**).The structure was further confirmed by mass spectrum,where molecular ion peak(M^+ at m/z 283.2910) corresponds to molecular mass (**4a**).

The structure of spiro compound **5a** was confirmed by the appearance of band at 3180 and 3185 (two>NH of imidazo ring),3195(NH of indole ring),1708 and1680 (CO of pyrimidine ring) cm^{-1}.^{1}H-NMR spectrum shows peaks at δ 3.65(NCH_3), 3.87(NCH_3), 6.25-7.06(aromatic protons), 8.05 and 8.56(two >NH imidazo ring) and 9.15 ppm due to NH of indolyl ring. ^{13}C-NMR spectrum exhibited peaks at δ 35.65(NCH_3),37.72(NCH_3),108.51(spiro carbon),110.90-145.80 (aromatic carbons), 186.05(CO of pyrimidine ring) and 189.09(CO of pyrimidine ring) ppm.Further,in the mass spectrum molecular ion peak appeared at (m/z) 299.2904 (M^+).

CONCLUSION:

An environmentally benign efficient and facile route is developed for the synthesis of novel indole derivatives. The reactions of indole-2,3-diones (**1**) with 5,6- diamino-1,3-dimethyl-(1*H*,3*H*) pyrimidinedione hydrochloride (**2**) have been investigated.Reacting (**1**) and (**2**) in water for 30 minutes only gave the hydrazone , 3-[5-(6-amino-1,3-dimethyl -2,4-(1*H*,3*H*) pyrimidinedione)]imino2(1*H*) ones (**3a-c**).When reaction time of reacting **1** and **2** in water increased to 4-5 hours the product obtained is 1,3-dimethyl-1*H*-indolo[3,2-g] pteridine-2,4-(3*H*,10*H*) diones (**4a-d**).If tetrabutyl ammonium bromide (TBAB) used as surfactant under aqueous micellar medium for reaction of **1** and **2** then product obtained is 4',6'-dimethyl spiro[3*H*-indole-3,2'(1'*H*)1,3-imidazo[4,5-d]pyrimidine]-2,5'.7'-triones (**5a-f**). The synthesized compounds were evaluated for antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* bacteria and can be used as potential broad-spectrum antibacterial agent.

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