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RESEARCH ARTICLE

SYNTHESIS, AND BIOLOGICAL EVALUATION OF SOMW NOVEL INDOLYL-PYRIMIDINE DERIVATIVES

Vaijinath A. Verma

Shri Prabhu Arts, Science & J. M. Bohra Commerce Degree College, Shorapur, Karnataka India

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ABSTRACT

A novel series of required intermediate 5-((5-substituted3-phenyl-1*H*-indol-2-yl) methyleneamino)-6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-diones (**3a-c**) was prepared on condensation 5,6-diamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**1**) with various 5- substituted 3-phenyl-1*H*-indole-2-carbaldehyde (**2a-c)** gave the respective Schiff bases, which on cyclization with thionly chloride afforded targeted compound 8-(5-substituted 3-phenyl-1*H*- indol-2-yl)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-diones (**4a-c)**. These newly synthesized compounds were screened for their antimicrobial and antioxidant activities, Compounds **3c**, **4a** and 4c exhibited promising antimicrobial activity. Compounds **3a**, **3b**, **4a** and **4b** showed potency of antioxidant activities.

INTRODUCTION

Heterocyclic nucleus imparts an important role in medicinal chemistry and serves as a key template for the development of various therapeutic agents. Pyramiding and its myriad derivatives have captured a unique attention in various pharmacological activities[1-5]. As a result pyrimidine has been subjected to a large variety of structural modifications in order to synthesise derivatives with better biological potency. The literature on pyrimidine derivatives, which occur in natural products[6].

The importance of the pyrimidine ring system as an antipurine and hence its importance as an anticancer agent has been previously recognized. It has wide antimicrobial activities including bacteriostatic, antiprotozoal, antischistosomal and AIDS[7-11]. On the other hand indole and its derivatives are known to possess a wide spectrum of biological activities such as antimicrobial[12], antioxidant[13], antiviral[14], anti-HIV[15], antimalarial[16], antituberculosis[17] anticancer agent[18], blocking carcinogenic substances before they reach their cellular targets and eliminating DNA damage in cell nuclei.

In animal models, prevents the development of malignancies, including cervical cancer,[19] breast cancer[20], prostate cancer,[21] endometrial cancer[22] and skin cancer.[23] It is a strong antioxidant and stimulators of detoxifying ezymes,[24] protecting the structure of DNA.[25]

MATERALS AND METHODS

General

All the reagents were obtained commercially and used by further purification. Melting points were determined by an open capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography using silica gel-G coated Al plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors, using benzene: ethylacetate (1:1) and/or tolune:ethylacetate (1:1). The IR (KBr pellet) spectra were recorded on a Perkin-Elmer (Spectrum ONE) FT-IR Spectrometer.

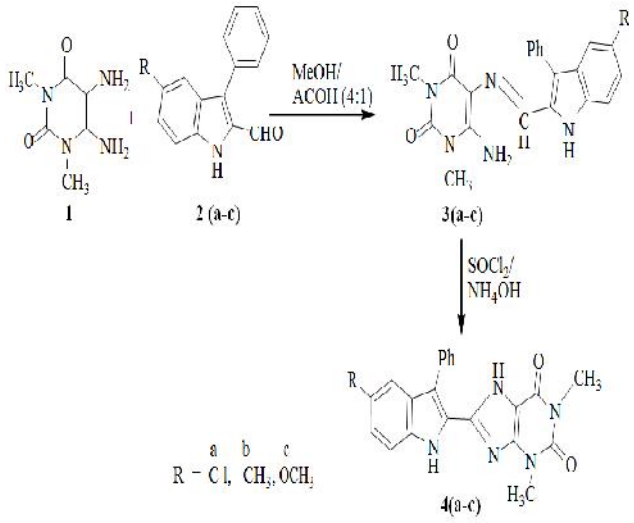
The ^{1}HNMR (DMSO-d6) spectra were recorded with a BRUKER NMR 500 MHz spectrometer the chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by Electron Impact method on JEOL GC mate spectrometer at 70 eV. Elemental analyses were performed on flash EA 1112 series elemental analyzer.

Chemistry

In the present work, the key intermediate 5-[(5-substituted-3-phenyl-1*H*-indol-2-yl) methyleneamino]-6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-diones (**3a-c**) afforded by 5,6- diamino-1,3-dimethylpyrimidine 2,4(1*H*,3*H*)-dione (**1**) using reported method [26,27] on

Corresponding author:* **Vaijinath A. Verma
Shri Prabhu Arts, Science & J. M. Bohra Commerce Degree College, Shorapur, Karnataka India

condensation with various 5-substituted 3-phenyl-1*H*-indole-2-carbaldehyde (**2a-c)** using reported procedure[28] in methanol and acetic acid (4:1). Compounds (**3a-c**) by refluxed in the thionylchloride for 30-40 min to get the final compounds 8-(5-substituted-3-phenyl-1*H*-indol-2-yl)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-diones(**4a-c**), respectively, (scheme-1)



Scheme-1 Schematic pathway for the synthesis of compounds **3-4(a-c)**

Synthesis

5,6-diamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (1) was by following literature procedure [26,27]

5-Substituted 3-phenyl-1*H*-indol-2-carboxaldehydes (2a-c) were prepared by literature method[28]

General procedure for the synthesis of various 5-[(5-Substutied-3-phenyl-1*H*-indol-2-yl) methyleneamino]-6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-diones (3a-c).

To a stirred solution of 5,6-Diamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**1)** (1.0g, 5.87 mol) in MeOH- AcOH (4:1 40 ml) was slowly added 5-substituted 3-phenyl-1*H*-indole-2-carbaldehyde (**2a-c)** in methanol (24 ml). The reaction mixture was further stirred overnight at room temperature. The residue obtained after removal of solvent under reduced pressure was dissolved in ice-cold water and alkalized with sodium hydroxide. The resultant turbid solution was cooled in ice for complete precipitation. The precipitate obtained was filtered off, washed with ice cold water and dried to obtained pure 3a-c.

5-[(5-chloro-3-phenyl-1H-indol-2-yl)methyleneamino]-6-amino-1,3- Dimethylpyrimidine-2,4(1*H*,3*H*)-dione 3a

Yield: 72 %, mp 214-215 ºC; FTIR (KBr) cm^{-1}: 3400, 3150 (indole-NH, NH_2); 1700, 1630 (C=O); 1616 (C=N); ^1H NMR (DMSO-*d6*, δ, ppm) 12.00 (s, 1H, indole NH); 8.80(s, 1H, N=CH); 7.20-8.00 (m, 10H, Ar-H); 6.24 (br s, 2H, NH_2); 3.30 (s, 3H, CH_3); 2.30(s,3H, CH_3); MS (EI) *m/z* 407 (M^+); 409 (M^++2). Anal. % $C_{21}H_{18}N_5O_2Cl$: C, 61.84; H, 4.45; N, 17.17; Found: C, 61.85; H, 4.45; N, 17. 18.

5-[(5-methyl-3-phenyl-1H-indol-2-yl)methyleneamino]-6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione 3b

Yield: 57%, mp 206-207 ºC; FTIR (KBr) cm^{-1}: 3332, 3214 (indole-NH, NH_2); 1697, 1640 (C=O); 1614 (C=N); ^1H NMR (DMSO-*d6*, δ, ppm) 12.01 (s, 1H, indole NH); 8.24 (s, 1H, N=CH); 6.90-7.90 (m, 11H, Ar-H); 6.50 (br s, 2H, NH_2); 3.25 (s, 3H, CH_3); 2.69 (s,3H, CH_3); 2.25 (s,3H, CH_3); Anal. % $C_{22}H_{21}N_5O_2$: C, 68.20; H, 5.46; N, 18.08. Found: C, 68.23; H, 5.47; N, 18.04.

5-[(5-methoxy-3-phenyl-1H-indol-2-yl)methyleneamino]-6-amino-1,3-dimethylpyrimidine-2,4(1H,3H)-dione 3c

Yield: 62%, mp 200-201 ºC; FTIR (KBr) cm^{-1}: 3235, 3204 (indole-NH, NH_2); 1680, 1640 (C=O); 1620 (C=N); ^1H NMR (DMSO-*d6*, δ, ppm) 11.91 (s, 1H, indole NH); 8.20 (s, 1H, N=CH); 7.10-8.10 (m, 8H, Ar-H); 6.10 (br s, 2H, NH_2); 3.10 (s, 3H, OCH_3); 2.69 (s,3H, CH_3); 2.35 (s, 3H, CH_3); Anal. % $C_{22}H_{21}N_5O_3$: C, 65.50; H, 5.25; N, 17.36. Found: 65.57; H, 5.29; N, 17.33.

General procedure for the synthesis of various 8-(5-substuted-3-phenyl-1*H*-indol-2-yl)-1,3- dimethyl-1*H*-purine-2,6(3*H*,7*H*)-diones 4(a-c).

Compounds (3a-c) (1.0g, 2.5 mol) obtained were refluxed separately in thionly chloride (20 ml) for 30-40 min to affect cyclization. The excess thionly chloride was removed under reduced procedure to obtain a solid product. Ice cold water was added to it and resultant suspension was neutralized with ammonium hydroxide solution. The precipitate obtained was collected by filtration, dried and recrystallized from a mixture of DMF and methanol to afford the desired products **4a-c**.

8-(5-Chloro-3-phenyl-1*H*-indol-2-yl)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione 4a

Yield: 68%, mp 245-246 ºC; FTIR (KBr) cm^{-1}: 3230 (indole-NH); 1720, 1630 (C=O); ^1H NMR (DMSO-*d6*, δ, ppm) 12.00 (s, 1H, indole NH); 7.00-8.00 (m, 9H, Ar-H); 3.40 (s, 3H, CH_3); 2.35 (s, 3H, CH_3); MS (EI) *m/z* 405 (M^+); 407 (M^++2). Anal. % $C_{21}H_{16}N_5O_2Cl$: C, 62.15; H, 3.97; N, 17.26. Found: C, 62.18; H, 3.95; N, 17.24.

1,3-dimethyl-8-(5-methyl-3-phenyl-1*H*-indol-2-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione 4b

Yield: 60%, mp 236-237 ºC; FTIR (KBr) cm^{-1}: 3350 (indole-NH); 1660, 1640 (C=O); ^1H NMR (DMSO-*d6*, δ, ppm) 11.59 (s, 1H, indole NH); 7.00-8.10 (m, 10H, Ar-H); 3.30 (s, 3H,

CH_3); 2.23 (s, 3H, CH_3); 2.50 (s, 3H, CH_3); Anal. % $C_{22}H_{19}N_5O_2$: C, 68.56; H, 4.97; N, 18.17. Found: 68.60; H, 4.95; N, 18.20.

8-(5-methoxy-3-phenyl-1*H*-indol-2-yl)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione 4c

Yield: 59%, mp 265-266 ºC; FTIR (KBr) cm^{-1}: 3322 (indole-NH); 1691, 1630 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.06 (s, 1H, indole NH); 7.20-8.00 (m, 10H, Ar-H); 3.20 (s, 3H, OCH_3);
2.80 (s, 3H, CH_3); 2.25 (s, 3H, CH_3); Anal. % $C_{22}H_{19}N_5O_3$: C, 65.83; H, 4.17; N, 17.45. Found: C, 65.85; H, 4.19; N, 17.44.

RESULT AND DISCUSSION BIOLOGICAL ACTIVITIES

Antimicrobial activities

The screening of newly synthesized compounds **3-4(a-c)** was carried out antibacterial activity against *Escherichia coli, Bacillus subtilis* and *Klebsiella pneumoniae* bacteria species and fungal species *Asperigillus niger, Asperigillus flavus and Asperigillus fumigates* by cup-plate method[29] using nutrient agar as medium. The holes of 6 mm diameter were punched carefully using a sterile cork borer and these were filled with test solution (1000 µg/ml in DMF) and DMF used as control. The plates were incubated at 37^0C for 24 h and 72 h in case antibacterial and antifungal activity, respectively. The diameter of the zone of inhibition for all the test compounds was measured and the results were compared with the standard drug streptomycin (Std_1) for bacterial activity and flucanazole (Std_2) for antifungal activity (**Table-1**).

Table 1 Antimicrobial activities for newly synthesis compounds 3, 4 (a-c)

Comp. No.	Diameter of zone of inhibition in mm[#]					
	Antibacterial Activity			Antifungal Activity		
	E. coli	B. *subtilis*	*K. pneumoniae*	A. *niger*	*A. flavus*	*A. fumigates*
3a	20	23	20	19	20	10
3b	19	15	15	19	13	15
3c	20	13	14	18	21	17
4a	22	24	21	18	20	17
4b	16	11	20	10	17	13
4c	21	13	19	19	21	18
Std_1	23	26	22	-	-	-
Std_2	-	-	-	20	22	19

Including diameter of well[#], control (DMF) = no activity, streptomycin (Std_1) and flucanazole (Std_2) were used as standards for antibacterial and antifungal activities, respectively.

Antibacterial result of the test compounds revealed that the compounds **3a**, **3c**, **4a** and **4c** exhibited good zone of inhibition against *E. coli*, **3a**, and **4a e**xhibited maximum zone of inhibition against *B. subtilis*, **3a**, **4a** and **4b** exhibited good zone of inhibition against *K. pneumonia*, whereas **4a** exhibited maximum zone of inhibition against all bacteria. Antifungal results indicated that the compounds **3a**, **3b**, **3c**, **4a** and **4c** exhibited maximum zone of inhibition against *A. niger*. Compounds **3a**, **3c**, **4a** and **4c** exhibited good zone of inhibition against *A. flavus*. **3c**, **4a** and **4c** exhibited maximum zone of inhibition against *A. fumigates***.** whereas, **3a**,**3c** and **4a** showed good zone of inhibition against all fungi. These results suggest that, compound **4a** exhibited good antibacterial activity against all the tested bacterial strain. This may be due to the electronegative nature of chlorine atom present at C-5 position of indole nucleus and 1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione system linked to position-2 of indole. Rest of the compounds in the series exhibited moderate to less activity. Compounds **3c** and **4c** showed potent antifungal activity against all tested fungi. This may be dueto the presence of electron donating methoxy group at position-5 of indole nucleus and 1,3- dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione system linked at position-2 of indole. Rest of the test compounds showed moderate to less activity against all tested fungal strains.

Antioxidant activity assay

1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Activity (RSA)

The free radical scavenging activity (RSA) of compounds **3-4(a-c)** at concentration (25, 50, 75 and 100 µg/mL) was carried out in the presence of freshly prepared solution of stable free radical DPPH (0.04% w/v) following Hatano's method[30] using 2-tert-butyl-4-methoxyphenol (butylated hydroxy anisole, BHA) and 2-(1,1-dimethylethyl)-1,4-benzenediol (2-tert. butyl hydroquinone, TBHQ) as standards. All the test analyses were performed on three replicates and results areaveraged. The results in percentage are expressed as the ratio of absorption decrease of DPPH in the presence test compounds and absorption of DPPH in the absence of test compounds at λ 517 nm on ELICO SL 171 Mini Spec spectrophotometer. The percentage scavenging activity of the
DPPH free radical was measured using the following equation.

$$\%\,DPPH\,Radical\,Scavenging = \frac{(Absorbance\,of\,control - Absorbance\,of\,test\,sample)}{(Absorbance\,of\,control)} \times 100$$

The results are illustrated in **Fig-1**. Compounds **3a** showed good RSA against DPPH. Indeed radicals were scavenged

71.18 % at 75 µ g/mL and 87.93% at 100 µ g/mL at concentrations.

Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) activity

The reducing power of the synthesized compounds **3-4(a-c)** was determined according to the literature method[31]. Different concentration of samples (25, 50, 75 and 100 µ g/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH=6.6) and potassium ferricyanide (2.5 mL, 1%).

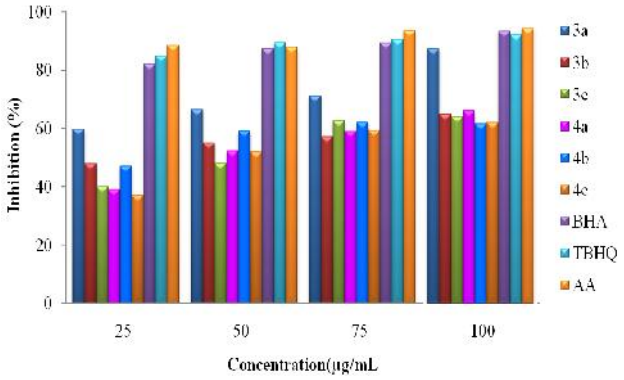


Fig. 1 RSA of the compounds 3-4(a-c)

The mixture was incubated at 50 °C for 20 min. After which a portion of trichloroacetic acid (2.5 mL, 10%) was added to the mixture and centrifuged for 10 min, at 1000 Xg. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1 %). Then absorbance at λ 700 nm was measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The analysis of results indicated that, compounds **3b** and **4b** exhibited good reducing activity at 100 µ g/ml concentration, whereas compound **4b** showed reducing power at 75 µ g/ml concentration. The results are shown in the
Fig-2.

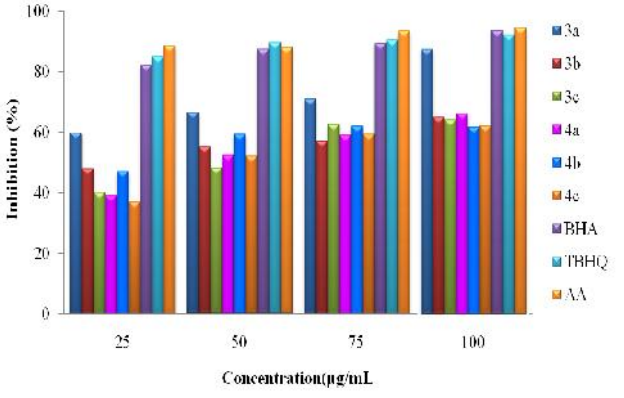


Fig. 2 Ferric (Fe3+) ions reducing capacity of compound 3, 4 (a-c).

Ferrous (Fe2+) metal ion chelating activity

The chelating activity of ferrous ions by synthesized compounds **3-4(a-c)** was estimated by following reported method[32]. The test samples (25, 50, 75 and 100 µ g/mL) in ethanolic solution (0.4 mL) were added to a solution of $FeCl_2$ (0.05 mL, 2 mM). The reaction was initiated by the addition of ferrozine (0.2 mL, 5 mM) and the total volume was adjusted to 4 mL with ethanol. Ferozine reacted with the divalent iron form stable magenta complex species that were very soluble in water. The mixture was shaken vigorously and kept at room temperature for 10 min. Then the absorbance of the solution was measured spectrophotometrically at λ 562 nm. All test analyses were run in triplicate and averaged. The percentage of inhibition of the ferrozine Fe^{2+}complex formations was calculated using the formula:

$$\text{Ferrous ion chelating effect (\%)} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

In this assay, synthesized compounds interfered with the formation of ferrous and ferrozinecomplex. From the **Fig-3,** it was conclude that compounds **3a** and **4b** exhibited good chelating activity and are able to capture ferrous ions before ferrozine.

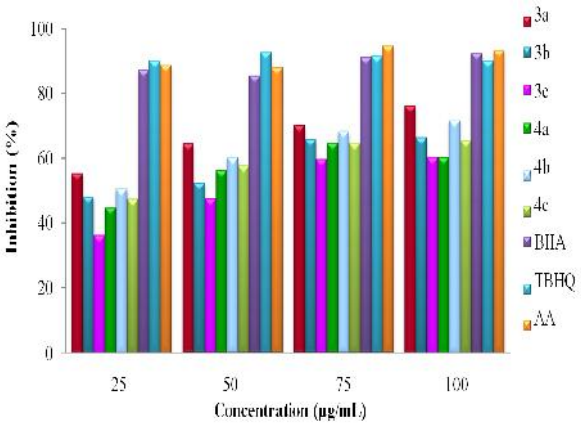


Fig. 3 Metal chelating activity of compounds 3-4(a-c)

CONCLUSION

The present study revealed that, the newly synthesized compounds having chloro and methoxy substitutions enhanced the antimicrobial activity, among the synthesized compounds, **3c**, **4a** and**4c** was found to be most active against all the microorganisms tested. Whereas, compound bearing chloro and methyl substitutions on indole nucules exhibited significant in antioxidant activities. Therefore, findings will provide great impact on chemist and biochemist for further investigations in this field.

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