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Original Research Article

Determination of radical scavenging activities of some pyrimidine derivatives

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Abstract

Purpose: *To synthesize some pyrimidine derivatives and investigate their radical scavenging activities.*
Methods: *A series of newly pyranopyrimidines derivatives and dithiopyridopyrimidinediones were synthesized by condensation of barbituric acid, malononitrile and different substituted benzaldehydes reacted with 1,4-Diazabicyclo[2.2.2] octane (DABCO) as a base. Compounds P1-7 (series 1), S1-11 (series 2) Scheme 1 and 6-amino-2-thiouracil with aromatic aldehydes in glacial acetic acid under reflux J1-13 (series 3) Scheme 2. ^{1}H & ^{13}C NMR, CHN, GC-MS and IR were used to characterize the compounds and were also screened for radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (IC_{50} = 50 µg/ml).*
Results: *The potency of radical scavenging activity was ranked as series 1 > series 3 > series 2. Compounds P3, J4, S10, P5, P7 with inhibitory concentration at 50 % level (IC_{50}) of 12, 40, 48, 50, and 54 µg/ml, respectively, showed radical scavenging activity equal or more potent than the standard antioxidant, ascorbic acid (IC_{50} = 50 µg/ml).*
Conclusion: *Series of newly pyranopyrimidines and dithiopyridopyrimidinediones derivatives have been successfully synthesized, and they demonstrate good radical scavenging activity.*

Keywords: *Pyranopyrimidine, Dipyrimidines, Anti-oxidant, DPPH, Ascorbic acid, Radical scavenging*

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INTRODUCTION

Pyrimidine is a six-membered heterocyclic aromatic organic compound that contains two nitrogen atoms at positions 1 and 3. It is known to possess some pharmacological activities, such as antiviral, anticancer [1-3], antimicrobial [4-6], anti-inflammatory [7] and antimalarial [8,9]. Molecules containing a pyrimidines scaffold is known to have pharmacological properties [10]. Some heterocyclic derivatives of pyrimidines such as pyranopyrimidines and dithiopyridopyrimidinediones derivatives contain pyrimidine, pyrano, pyrido and aryl moieties [11].

The pyranopyrimidines and dithiopyridopyrimidinediones derivatives have been previously

synthesized by various methods as previously reported. [12].

Reactive oxygen species (ROS), such as superoxide radical anions, hydrogen peroxide and hydroxyl radical are free radicals continuously produced during normal metabolic pathways and in pathology [13]. Excessive production of ROS is deleterious to cellular proteins and lipids due to oxidative damage. The body's endogenous antioxidant system acts as a ROS scavenger to protect cells and macromolecules from oxidative damage. Disturbances of anti-oxidant status are seen in diabetes, hypertension, obesity, infertility, Alzheimer's disease, Parkinson's disease and cancer [14]. As such, supplementing the endogenous antioxidant system is being investigated as a possible therapy in prevention, as well as limiting progression and development of complications, of chronic diseases. In view of this, we report on the investigation of the antioxidant properties of the synthesized pyrimidine compounds using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and free radical scavenging assay.

EXPERIMENTAL

Materials

All chemicals and solvents used in this study are of analytical grade and were purchased from Sigma Aldrich via Capital Laboratories, South Africa. All organic solvents were redistilled and dried according to standard procedures. Nuclear magnetic resonance (NMR) spectra were recorded using Bruker Avance III 400 MHz spectrometer at room temperature, with chemical shifts (δ) recorded against the internal standard, tetramethylsilane (TMS). IR spectra were recorded on a Perkin Elmer Spectrum 100 ATR-FTIR spectrometer. For GC-MS analyses, the samples were analyzed on an Agilent GC-MSD apparatus equipped with DB-5SIL MS (30m ×0.25mm i.d., 0.25 μm film thickness) fused-silica capillary column. Helium (at 2mL min−1) was used as a carrier gas. MS was operated in EImode at 70 eV. Optical rotation was recorded using a polarimeter (Model 341, Perkin Elmer Inc, USA). Melting points were recorded on an Ernst Leitz Wetzlar microhot stage melting point apparatus.

General procedure for the synthesis of pyranopyrimidione derivatives (P1-7, S1-11)

Reported procedure [12]. was used in synthesizing this library of compounds by reacting barbituric acid, benzaldehyde derivatives (ArCHO), malononitrile (1.0 mmol each) and 10% DABCO in a clean 50ml round bottom flask containing 20ml of aqueous ethanol (Scheme 1). The reaction mixture was stirred for a period of 60 minutes. The progress of the reaction was monitored by TLC. For most of this synthesis, the products were filtered, recrystallized in ethanol and dried under vacuum pressure, with good yields of the final products.

General procedure for the synthesis of dithiopyridopyrimidinediones) derivatives (J1-13)

A solution of 6-amino-2-thiouracil 4 (3 mmol) in glacial acetic acid (15 ml) and 0.5 equiv. of the appropriate aromatic aldehyde was heated under reflux for 4 hours. The reaction mixture was diluted with water and then allowed to cool to room temperature. The crude product was collected and recrystallized from a suitable solvent. Details have been reported in previously published articles from our group [15].

Evaluation of antioxidant properties

The free radical scavenging activity of the synthesized compounds was determined by 1, 1-diphenyl-2- picrylhydrazyl (DPPH) method, as described by Brand William et al (1995) [16]. In this protocol, DPPH• acts as a stable free radical with a purple colour. In the presence of an antioxidant that donates an electron, the purple colour of DPPH• decays. This change can be monitored spectrophotometrically and used to assess radical scavenging activity, which equates to antioxidant activity of compounds. For this study, compounds were dissolved in a minimum volume of DMSO and diluted in ethanol to 1mg/ml. To 1ml of compound or ascorbic acid, the standard (500, 250, 125 and 62.5 µg/ml) in a test tube, 3 ml of 0.5 mM ethanolic solution of DPPH was added. The mixture was vortexed vigorously and incubated for 30 minutes in the dark at room temperature. Absorbance was read spectrophotometrically at 517 nm and inhibition of DPPH free-radical scavenging activity (D) was computed as in Eq 1.

D {(Ac As)/Ac}100 …….. (1)

where Ac and As are the absorbance of control and test sample, respectively.

RESULTS

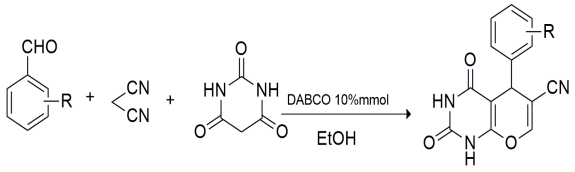
Chemistry

Various pyranopyrimidiones derivatives P1-7 and S1-11) (Scheme 1) have been reported in the

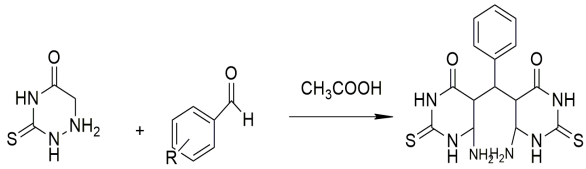
literature [12]. Scheme 1 showed the good yields of the synthesized final products, whereas, Scheme 2 revealed dithiopyridopyrimidines derivatives (**J1-13**) synthesized, as reported in the literature [15]. Hence, the formation of the title compounds was confirmed and further established by ^{13}C NMR and mass spectroscopic studies, which agree with the molecular formula.

Antioxidant activity

Radical scavenging activity of compounds is shown in Tables 1 to 4. Better radical scavenging activity was observed for series 1 than for series 2 compounds, with three (27%) showing no activity at the lowest concentration of 62.5µg/ml, contrary to series 2 that showed three (27%) with no activity at all and five (45%) showing no activity at the lowest concentration of 62.5µg/ml. Although series 3 compounds showed radical scavenging activity at all concentrations, the IC_{50} values were very high compared to that in series 1. Thus, potency in radical scavenging activity is ranked as series 1 > series 3 > series 2 (Table 1). Overall, when compared to ascorbic acid (IC_{50} = 50µg/ml), a known and potent antioxidant compound, five compounds have equal or better radical scavenging activity: 3, C4, B10, 5, 7 with IC_{50} of 12, 40, 48, 50, and 54 µg/ml respectively (Table 2 and Figure 1).



Scheme 1: Synthesis of pyranopyrimidiones (P1-7, S1-12) (oxygenated and halogenated compounds)



Scheme 2: Synthesis of dithiopyridopyrimidiones derivatives (J1-13)

Table 1: Radical scavenging activity of synthesized compounds

Compound	DPPH inhibition (%)				
	500 µg/ml	***250 µg/ml***	***125 µg/ml***	***62.5 µg/ml***	***IC_{50} (µg/ml)***
P1	42	28	18	11	595
P2	66	52	32	17	240
P3	79	79	78	78	12
P4	47	31	17	-	532
P5	78	77	73	62	50
P6	87	54	26	17	230
P7	81	82	77	57	54
S1	60	45	28	16	225
S2	42	25	18	-	595
S3	28	19	12	-	893
S4	50	32	17	-	500
S5	-	-	-	-	1000
S6	45	26	-	-	555
S7	-	-	-	-	1000
S8	-	-	-	-	1000
S9	25	11	-	-	893
S10	28	28	28	18	900
S11	88	88	77	65	48
J1	87	65	47	33	133
J2	91	89	69	51	61
J3	57	44	34	29	440
J4	91	91	89	78	40
J5	65	50	35	28	250
J6	82	69	46	39	136
J7	54	38	31	26	460
J8	59	46	32	25	420
J9	52	44	34	27	480
J10	63	46	33	24	270
J11	50	40	35	27	500
J12	52	39	30	23	480
JP13	64	44	33	21	280
Ascorbic acid	96	96	92	65	50

Table 2: All compounds DPPH inhibition activity ranked (highest to lowest) comparing with AA

Compound	DPPH inhibition (%)				
	500 µg/ml	***250 µg/ml***	***125 µg/ml***	***62.5 µg/ml***	***IC50 (µg/ml)***
P3	79	79	78	78	12
J4	91	91	89	78	40
S10	88	88	77	65	48
P5	78	77	73	62	50
Ascorbic acid	96	96	92	65	50
P7	81	82	77	57	54
J2	91	89	69	51	61
J1	87	65	47	33	133
J6	82	69	46	39	136
S1	60	45	28	16	225
P4	57	54	38	11	230
P2	66	52	32	17	240
J5	65	50	35	28	250
J8	68	50	41	33	250
J4	56	41	28	17	446
J10	53	44	35	26	472
S4	50	32	17	-	500
J11	50	40	35	27	500
B7	45	26	-	-	555
P1	42	28	18	11	595
S2	42	25	18	-	595
J9	42	39	30	27	595
P6	41	22	12	-	610
S3	28	19	12	-	893
S9	25	11	-	-	893
S5	-	-	-	-	1000
S7	-	-	-	-	1000
S8	-	-	-	-	1000

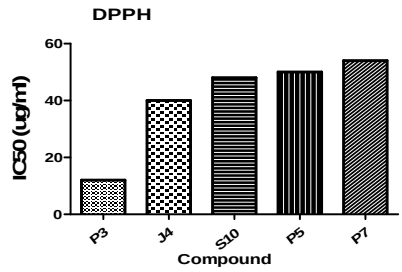


Figure 1: Potential inhibition capacities of the most potent synthesized compounds (***rank order:*** P3 > J4> S10>P5 > P7)

DISCUSSION

A series of new pyranopyrimidiones and dithiopyridopyrimidiones derivatives were synthesized and evaluated for radical scavenging activity.

Pyrimidines, also known as the breakdown products of uric acid, play a vital role in the medicinal chemistry, due to their structures and composition, and act as the key templates associated with the development of different therapeutic agents [16,20]. Several literatures have reported on antioxidant activities of some pyrimidine derivatives [17,18]. In the present study, five compounds have equal or better radical scavenging activity when compared to ascorbic acid (IC_{50} = 50µg/ml), a known and potent antioxidant compound. This may be due to the available NH and SH group present in the synthesized pyrimidines [19].

CONCLUSION

A series of pyranopyrimidiones and dithiopyridopyrimidiones derivatives have successfully been synthesized and they exhibit radical scavenging activity in the rank order of series 1 > series 3 > series 2.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) mentioned in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Oluwole S. Aremu and Neil Koorbanally devised and designed the study, Oluwole S. Aremu carried out the laboratory work on the chemistry aspect, Olukayode O. Aremu and Constance R. Sewani-Rusike investigated the antioxidant properties of the synthesized pyrimidines, Lebogang Katata-Seru edited the raw data. The manuscript was proof-read by all the authors and approved for publication.

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