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Research Article

MICROWAVE ASSISTED SYNTHESIS OF NOVEL PYRIMIDINES BEARING BENZENE SULFONAMIDES AND EVALUATION OF ANTICANCER AND ANTIOXIDANT ACTIVITIES

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ABSTRACT

A series of some new pyrimidine containing sulfonamides moieties were synthesized via the reaction of chalcones with sulfaguanidine by conventional and microwave oven synthesis. And been evaluated for their anticancer and antioxidant activities. All structures of the compounds were elucidated by elemental analysis and spectral datas.

Keywords: Chalcones, Pyrimidines, Anticancer Activity, Antioxidant Activity.

INTRODUCTION

Pyrimidine derivatives have attracted a great deal of interest owing to their medicinal activities like anticancer[1], antiviral[1], antitumor[2], anti-inflammatory[2,3,4] analgesic[2 3] antioxidant[4] and others.

On the other, Sulfonamides constitute an important class of drugs. They possess various types of pharmacological activities such as antibacterial[1], high-ceiling diuretic[1], hypoglycemic[1], antithyroid[1], anti-inflammatory[5], and antiglaucoma[5].It is also known that aryl/heteroaryl sulfonamides may act as antitumor agents through perturbation of cell cycle in the G1 phase, distribution of microtubule assembly or angiogenesis inhibition[6]. Moreover, numerous sulfonamides were found to act as antitumor agents through carbonic anhydrase (CA) inhibition[7].

All these findings encouraged us to explore the synthesis of pyrimidines containing sulfonamides moieties and examine their activities as antitumor and antioxidant agents. Herein we report their microwave and conventional synthesis, antitumor evaluation against Ehrlich ascites carcinoma (EAC) cell lines and antioxidant evaluation using DPPH and nitric oxide scavenging method.

MATERIALS AND METHODS

Melting points were determined by capillary method and were uncorrected. The IR spectra is recorded by using Shimadzu Perkin Ekmer 8201 Pc IR Spectrometer using a thin film on potassium bromide pellets techniques and frequencies are expressed in cm^{-1}. The PMR spectra were recorded on Bruker Avance II 400 NMR spectrometer. All spectra were obtained in $CDCl_3$ and DMSO. Chemical shift values are reported as values in ppm relative to TMS (δ=0) as internal standard. The FAB mass spectra were recorded on JEOL SX-102/DA-6000 Mass spectrometer using Argon/Xenon (6Kv, 10Ma) as the FAB gas. The elemental analyses have been obtained using Vairo Elementar Model, CHN analyser and the results were found to be within ±0.4%. Microwave experiments were carried out on a microwave reactor (Cata-R, Catalyst Systems 140-700 W).

General Methods of Synthesis of Substituted Chalcones (JC1-JC8):

Synthesis of 3-(anthracenyl-9-yl)-1-(aryl substituted) prop-2-en-1-one

A mixture of 9-anthraldehyde (0.01 mol) and substituted acetophenones (0.01 mol) in ethanol (20 ml) were stirred together for 24 h, in presence of 20% NaOH (4 ml). The mixture was poured into crushed ice and acidified with 5%HCl. The product obtained was filtered, washed with water and re-crystallized from ethanol[8].

3-(anthracenyl-9-yl)-1-(4-bromophenyl) prop-2-en-1-one (JC1)

IR (cm^{-1}): 3040 (CH str), 1694 (α,β unsaturated keto group), 1509 (C=C Str), 668 (C-Br).

^{1}H NMR (δ ppm): 7.26-7.30 (d, 1H, =CH), 7.98-8.01(d, 1H, =CH), 7.551- 8.293 (m,13H, Ar-H)

Mass (m/z) (M^+) 387, (M^+ 2) 389.

Anal. Calcd for C, 71.33; H, 3.90, Found: C, 71.36; H, 3.87.

3-(anthracenyl-9-yl)-1-(4-fluorophenyl) prop-2-en-1-one**(**JC2)

IR (cm^{-1}): 3041, 3007 (CH str), 1696 (α,β unsaturated keto group), 1504 (C=C Str), 1338(C-F).

^{1}H NMR (δ ppm): 7.16-7.20 (d, 1H, =CH), 7.50-7.58(d, 1H, =CH), 7.54- 8.27 (m, 13H, Ar-H)

Mass (m/z) (M^+ 1) 327.
Anal. Calcd for C, 84.64; H, 4.63, Found: C, 84.60; H, 4.65.
3-(anthracenyl-9-yl)-1-(4-nitrophenyl) prop-2-en-1-one**(**JC3)

IR (cm^{-1}): 3125 (CH str), 1675 (α,β unsaturated keto group), 1510 (C=C Str), 1513 (C-NO_2).

^{1}H NMR (δ ppm): 7.70-7.74 (d, 1H, =CH), 8.14-8.16 (d, 1H, =CH), 7.55- 8.70 (m, 13H, Ar-H)

Mass (m/z) (M^+1) 353.

Anal. Calcd for C, 78.17; H, 4.28, Found: C, 78.19; H, 4.26.

General Methods of Synthesis of Substituted Pyrimidines (PS1-PS8):

Conventional method:

Synthesis of 4-amino-N-(4-(anthracen-9-yl)-6-(aryl substituted) pyrimidin 2yl)benzene sulfonamide

To chalcone (0.01 mol), sulfaguanidine (0.01 mol), and 20 ml dimethyl sulfoxide was added. The mixture was warmed and anhydrous potassium carbonate was added until solution became alkaline to litmus. The reaction mixture was refluxed for 12 hrs, cooled and poured into ice, stirred for 1 hr and left to stand overnight. The reaction mixture was cooled and poured into crushed ice and acidified with dilute acetic acid. The solid thus

obtained, was washed with water and recrystallized from ethanol/ethyl acetate[9]. Ethyl acetate: Acetone (9:1) is the solvent system for TLC. The physical datas are given in Table 1.

Microwave irradiation method

To chalcone (0.01 mol), sulfaguanidine (0.01 mol), and 20 ml dimethyl sulfoxide and anhydrous potassium carbonate was added. The reaction mixture was subjected to microwave irradiation. The reaction mixture was cooled and poured into crushed ice and acidified with dilute acetic acid. The solid thus obtained, was washed with water and recrystallized from ethanol/ethyl acetate. Ethyl acetate: Acetone (9:1) is the solvent system for TLC [10].

4-amino-N-(4-(anthracen-9-yl)-6-(4-bromophenyl)pyrimidin-2yl) benzene sulfonamide (PS1)

IR (cm^{-1}): 3403 (aromatic NH str), 3096 (aromatic CH str), 1566 (C=C Str), 1347 (SO_2), 635 (C-Br).

^{1}H NMR (δ ppm): 3.90 (s, 1H, NH), 6.982 (d, 2H, NH_2), 7.119-7.965 (m, 18H, Ar- H).

Mass (m/z): (M^+) 581, (M^+ 2) 583.
Anal. Calcd for C, 61.97; H, 3.64, Found: C, 61.98; H, 3.63
4-amino-N-(4-(anthracen-9-yl)-6-(4-nitrophenyl)pyrimidin-2yl) benzene sulfonamide (PS2)

IR (cm^{-1}): 3417 (aromatic NH str), 3056 (aromatic CH str), 1562 (C=C Str), 1356 (SO_2), 1432 (C-NO_2).

^{1}H NMR (δ ppm): 4.02 (s, 1H, NH), 6.97 (d, 2H, NH_2), 7.12-8.34 (m, 18H, Ar- H).

Mass (m/z): (M^+) 547.
Anal. Calcd for: C, 65.80; H, 3.87, Found: C, 65.82; H, 3.86.
4-amino-N-(4-(anthracen-9-yl)-6-(4-chlorophenyl)pyrimidin-2yl) benzene sulfonamide (PS3)

IR (cm^{-1}): 3098 (aromatic NH str), 3098 (aromatic CH str), 1509 (C=C Str), 1354 (SO_2), 732 (C-Cl).

^{1}H NMR (δ ppm): 4.21 (s, 1H, NH), 6.89 (d, 2H, NH_2), 7.04-8.05 (m, 18H, Ar- H).

Mass (m/z): (M^+ 2) 537.
Anal. Calcd for C, 67.09; H, 3.94, Found: C, 67.08; H, 3.96
4-amino-N-(4-(anthracen-9-yl)-6-(4-flurophenyl)pyrimidin-2yl) benzene sulfonamide(PS4)
IR (cm^{-1}): 3411 (aromatic NH str), 3013 (aromatic CH str), 1545 (C=C Str), 1332 (SO_2), 1348 (C-F).

^{1}H NMR (δ ppm): 4.15 (s, 1H, NH), 6.77 (d, 2H, NH_2), 7.19-7.98 (m, 18H, Ar- H).

Mass (m/z): (M^+ 2) 520.
Anal. Calcd for: C, 69.22; H, 4.07, Found: C, 69.20; H, 4.09.

Anti Tumor Activity by Tryphan Blue Dye Exclusion Method[11,12]**:**

The synthesized compounds were tested for their cytotoxicity in vitro, in comparison with 5- fluorouracil as reference drug, against EAC cells. EAC cells (1×10^6) were incubated with synthesized compounds at various concentrations of 25, 50, 100, 200µg/ml, in 1ml phosphate buffered saline(incorporated with 10µL DMSO) at 37°C for 3 hr. Viable cells were counted in a haemocytometer using the tryphan blue dye exclusion method. Experiments were carried out in triplicate. The results are given in table 3.

Evaluation of Antioxidant Activity:

DPPH Radical Scavenging Activity[13]

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored ethanol solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent.1 ml of the compounds containing concentrations (10-200 µg/ml) was mixed with 3 ml of 0.1mM solution of DPPH. The mixture was kept in dark for 30 minutes. After a 30 mins incubation period at room temperature, the absorbance was read against blank at 517 nm.

Nitric Oxide Free Radical Scavenging Method[13]**:**

Nitric oxide radicals (NO) were generated from sodium nitroprusside. 1 ml of sodium nitroprusside (10 mM) and 1.5 ml of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (10-200 µg/ml) of the test compounds and incubated for 150 mins at 25^{0}C and 1 ml of the reaction mixture was treated with 1 ml of Griess reagent. The absorbance of the chromatophore was measured at 546 nm. The results were expressed for both antioxidant methods as percentage of inhibition, which was calculated according to the following equation,

% inhibition = Control absorbance – Test absorbance x 100

Control absorbance

RESULTS AND DISCUSSION

Microwave irradiation of an equimolar mixture of chalcones and sulfaguanidine in DMSO and catalytic amounts of anhydrous potassium carbonate afforded the desired pyrimidines in good yields (72-84%). Although reactions were initially tried under conventional heating using DMSO as solvent, they took prolonged reaction times (12h) resulting in low yields (32-69%) (Figure 1).

Table 1: Physical Datas of Synthesized Chalcones

Chalcone No:	Compound	Physical State	Molecular Formula	Mol. Wt	M.P (°C)
JC1	4 –Br	Yellow Crystals	$C_{23}H_{15}BrO$	387	80-82
JC2	4 – F	Light yellow Crystals	$C_{23}H_{15}FO$	326	83-85
JC3	4–NO_2	Bright red Crystals	$C_{23}H_{15}NO_3$	353	104-106
JC4	4–Cl	Yellow Crystals	$C_{23}H_{15}ClO$	342	87-89
JC5	4 - NH_2	Light yellow Crystals	$C_{23}H_{17}NO$	228	96-98
JC6	3-Br	Light brown Crystals	$C_{23}H_{15}BrO$	387	88 -90
JC7	3–Cl	Light yellow Crystals	$C_{23}H_{15}ClO$	342	92-95
JC8	3-NO_2	Red Crystals	$C_{23}H_{15}NO_3$	353	111-112

Table 2: Physical Datas of Synthesized Pyrimidine Derivatives

Pyrimidines No:	R	Physical State	Molecular Formula	Mol. Wt	M.P (°C)	% yield
PS1	4 –Br	Brown Crystals	$C_{30}H_{21}BrN_4O_2S$	580	196-198	69
PS2	4 –NO_2	Brownish red Crystals	$C_{30}H_{21}N_5O_4S$	547	112-114	67
PS3	4-Cl	Bright red Crystals	$C_{30}H_{21}ClN_4O_2S$	536	104-106	62
PS4	4–F	Light red Crystals	$C_{30}H_{21}FN_4O_2S$	520	156-158	64
PS5	4 - NH_2	Brown Crystals	$C_{30}H_{22}N_5O_2S$	517	164-166	50
PS6	4-OH	Light brown Crystals	$C_{30}H_{22}N_4O_3S$	518	104-106	38
PS7	4-OCH_3	Dark brown Crystals	$C_{31}H_{24}N_4O_3S$	532	166-169	32
PS8	3-Br	Red Crystals	$C_{30}H_{21}BrN_4O_2S$	580	186-188	55

Table 3: Reaction time and yield of conventionally and microwave assisted synthesis of Pyrimidines

Compounds	Conventional synthesis		Microwave assisted synthesis	
	Time (h)	Yield (%)	Time (min)	Yield (%)
PS1-PS8	12	52-75	5-6	72-84

Table 4: Cytotoxicity Activities of Substituted Pyrimidines (PS1-PS8) by Tryphan Blue Exclusion Method

Compound	R	No. of dead cells (%) at different concentrations(µg/ml)			
		20	50	100	200
Control		1			
PS1	4 –Br	15	20	45	64
PS2	4 –NO_2	21	41	58	80
PS3	4-Cl	12	24	48	68
PS4	4–F	16	29	42	66
PS5	4 - NH_2	20	39	55	78
PS6	4-OH	11	20	41	65
PS7	4-OCH_3	14	34	42	73
PS8	3-Br	22	40	55	79
Standard	5-Fluorouracil	35	50	90	98

Table 5: Antioxidant Activity of Substituted Pyrimidines (PS1-PS8) by DPPH Radical Scavenging Activity

Compound	R	(% inhibition) at different concentrations(µg/ml)			
		10	50	100	200
PS1	4 –Br	41	50	63	65
PS2	4 –NO_2	59	**59**	**75**	**90**
PS3	4-Cl	32	45	54	68
PS4	4–F	54	**60**	**74**	**88**
PS5	4 - NH_2	43	52	67	73
PS6	4-OH	45	**58**	**71**	**89**
PS7	4-OCH_3	42	55	64	71
PS8	3-Br	43	55	64	72
Standard	Ascorbic acid	65	83	88	90

Table 6: Antioxidant Activity of Substituted Pyrimidines (PS1-PS8) by Nitric Oxide Free Radical Scavenging Method

Compound	R	(% inhibition) at different concentrations(µg/ml)			
		10	50	100	200
PS1	4 –Br	46	59	67	75
PS2	4 –NO_2	54	66	76	85
PS3	4-Cl	60	74	88	89
PS4	4–F	50	57	74	81
PS5	4 - NH_2	55	52	67	73
PS6	4-OH	49	56	61	70
PS7	4-OCH_3	43	51	64	78
PS8	3-Br	42	51	56	62
Standard	Ascorbic acid	65	83	88	90

In vitro anticancer studies for the synthesized Pyrimidines revealed that compounds PS2, PS5 and PS8 induced the greatest effect on EAC cells and they exhibit good anticancer action.

In vitro antioxidant studies for the synthesized Pyrimidines revealed that compounds PS2 and PS4 showed good radical scavenging activity in both methods when compared with the standard drug ascorbic acid. Further Table 5 and 6 indicate that radical scavenging activity in DPPH and nitric oxide methods increases with concentration.

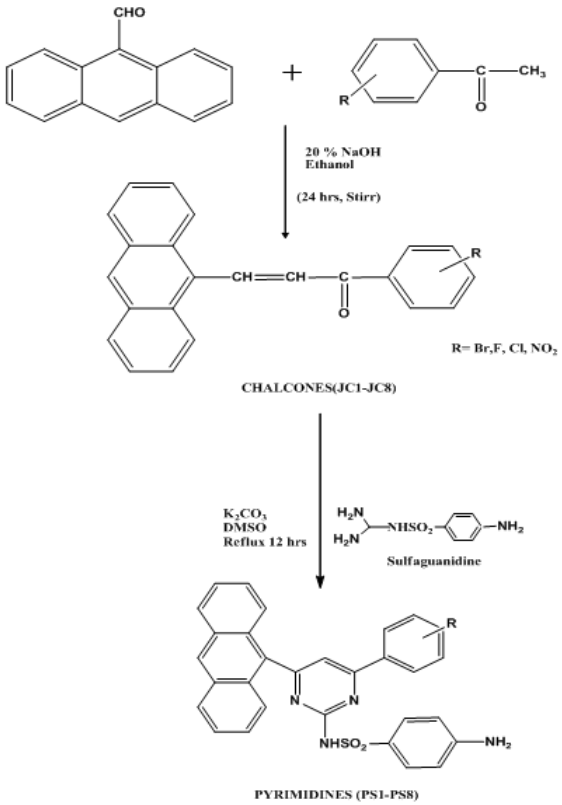

Figure 1: Reaction Scheme

CONCLUSIONS

The microwave-assisted synthesis of novel Pyrimidines has been performed. These compounds were obtained in good yields and confirmed by IR, NMR, MASS and Elemental spectral analysis. Biological activity studies showed that compounds exhibited significant antitumor and antioxidant activities.

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