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Original Article

SYNTHESIS OF NOVEL MERCAPTO-PYRIMIDINE AND AMINO-PYRIMIDINE DERIVATIVES OF INDOLINE-2-ONE AS POTENTIAL ANTIOXIDANT & ANTIBACTERIAL AGENTS

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ABSTRACT

A series of novel 1-(2mercapto-6-(substituted phenyl) pyrimidine-4-yl)-3-(2-substituted phenyl imino) indolin-2-one, and a series of novel1-(2 amino-6-(substituted phenyl)pyrimidine-4-yl)-3-(2-substituted phenyl imino)indolin-2-one were synthesized from different substituted chalconised Indole 2,3 dione. The structure of the compounds were elucidated by elemental and spectral (IR, ^{1}H-NMR and MS) analysis. The synthesized compounds were screened for their antioxidant activity by reducing power method and in vitro antimicrobial activity against the Gram-positive bacteria Staphylococcus aureus and Bacillus subtilis, the Gram negative bacteria Salmonella typhi, Shigella dysenteriae, Pseudomonas mirabilis and E.coli by cup plate agar diffusion method .Evaluation of the compounds revealed remarkable antioxidant activity and also very good antibacterial activity observed.

Keywords: Mercapto-Pyrimidine, Amino-Pyrimidine, Isatin, Antioxidant, Antibacterial

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1.0 INTRODUCTION

Isatin (1-*H* indole 2-3 dione) is synthetically versatile substance which is employed for the synthesis of large variety of heterocyclic compounds including some drugs. Over the years the molecules with isatin shows diversified biological activities, e.g. antibacterial[1], anticonvulsants[2], antiviral[3], antitubercular[4], activities. Due to interesting activity of various substituted pyrimidines as biological agents[5, 6,7,8], considerable attention has focused on this class and in continuation of our research work on synthesis of biologically active heterocyclic compounds, we investigated the synthesis of some novel mercapto-pyrimidines and amino-pyrimidines from chalconised isatin derivative. The antioxidant and antibacterial activities of the synthesized compounds are reported. Twelve compounds were synthesized, tested for reducing power and against gram positive, gram negative bacteria at 100 µg/ml. The structures of all compounds were confirmed on the basis of spectral data and elemental analysis.

2.0 Materials and method:

Melting points of the synthesized compounds were recorded in open capillary tubes and are uncorrected .The I.R spectra are recorded on a SHIMADZU-FT-IR spectrophotometer in KBr. The ^{1}H-NMR spectra was recorded on a Bruker-400 MHz spectrophotometer (DMSO-d_6) using TMS as internal standard and chemical shifts are expressed in δ ppm. A mass spectrum was recorded in Maldi MS shows m/z peak at 465. Purity of the compounds was checked by the TLC. For antibacterial activity the microbial strains are taken from Jadavpur University Kolkata, West Bengal. The characterizations of the synthesized compounds were given in Table - 1. The title compounds were prepared using the general synthetic strategy for the preparation of mercapto-pyrimidine and amino-pyrimidine derivatives from the chalcones described as follows.

(i) General preparation for the synthesis of 1-(2mercapto-6-(substituted phenyl) pyrimidine-4-

yl)-3-(2-substituted phenyl imino) indolin-2-one: ($7a_1$-$7a_3$) ($7b_1$-$7b_3$)

Different chalconised derivatives (0.01 mol), and thiourea were dissolved in absolute alcohol (20ml), few drops of concentrated hydrochloric acid were added and the reaction mixture was reflux [8] and the reaction was monitored by TLC. After completion of the reaction, it was poured in to 250 ml of ice cold water and kept for some time. The crude solid was filtered, dried and recrystallised with chloroform-ethanol mixture (1:2).

$7a_1$: 1-(2mercapto-6-(2-Nitrophenyl) pyrimidine-4-yl)-3-(2-Nitrophenyl imino) indolin-2-one; IR ($KBr.cm^{-1}$): 1330(Ar-NO_2), 1630(C=N), 822(S-H), 3403 (N-H Str), 1245(C-N), 896(CH=CH of aroma). ^1H NMR (300 MH_Z, DMSO-d_6, TMS, δ ppm): 12.8(s, 1H, SH), 6.8-8.0(m, Ar-H), 2.3-2.5, 3.4-3.6 and 4.7-5.2 (dd, pyrimidine ring.). MS: m/z : 498.07(M^+).

$7a_3$: 1-(2mercapto-6-(4-methoxyphenyl) pyrimidine-4-yl)-3-(2-Nitrophenyl imino) indolin-2-one; IR($KBr.cm^{-1}$):1330(Ar-NO_2), 1630(C=N), 822(S-H), 3403 (N-H Str),1245(C-N), 2808 (Ar-OCH_3), 896(CH=CH of aroma). ^1H NMR (300 MH_Z, DMSO-d_6, TMS, δ ppm): 12.8(s, 1H, SH), 3.8 (s, 3H, OCH_3), 6.8-8.0(m, Ar-H), 2.3-2.5, 3.4-3.6 and 4.7-5.2 (dd, pyrimidine ring.). MS: m/z :483.10(M^+).

$7b_1$: 1-(2mercapto-6-(2-nitrophenyl) pyrimidine-4-yl)-3-(2-Chlorophenyl imino) indolin-2-one;

IR($KBr.cm^{-1}$): 1333(Ar-NO_2) ,1636(C=N), 645(C-Cl)1740(C=O), 3048 (N-H Str),1245(C-N), 824(S-H), 890(CH=CH of aroma). ^1H NMR (300 MH_Z, DMSO-d_6, TMS, δ ppm): 12.8(s, 1H, SH), 6.89-7.6 (8H,m,2Ar-H), 2.4-2.5, 3.4-3.6 and 4.5-5.0 (dd, pyrimidine ring.). MS: m/z 487.05(M^+)

$7b_3$: 1-(2mercapto-6-(4-methoxyphenyl) pyrimidine-4-yl)-3-(2-Chlorophenyl imino) indolin-2-one; IR($KBr.cm^{-1}$): 2808 (Ar-OCH_3),1630(C=N), 824(S-H), 648(C-Cl),1734(C=O), 3034 (N-H Str), 1245(C-N) 880(CH=CH of aroma). ^1H NMR (300 MH_Z,

DMd_6, TMS, δ ppm): 12.6(s, 1H, SH), 3.8 (s,3H, OCH_3), 6.89-7.6 (8H,m,2Ar-H), 2.0-2.4, 3.2-3.4 and 4.4-4.9 (dd, pyrimidine ring.). MS: m/z 472.08(M^+).

(ii) General preparation for the synthesis of 1-(2-amino-6-(substituted phenyl) pyrimidine-4-yl)-3-(2-substituted phenyl imino) indolin-2-one: ($8a_1$-$8a_3$) ($8b_1$-$8b_3$)

Different chalconised derivatives (0.01 mol), and guanidine hydrochloride (0.01 mol) were dissolved in absolute alcohol (20ml), few drops of concentrated hydrochloric acid were added and the reaction mixture was reflux [8] and the reaction was monitored by TLC. After completion of the reaction, it was poured in to 250 ml of ice cold water and kept for some time. The crude solid was filtered, dried and recrystallised with rectified spirit.

$8a_1$: 1-(2-amino-6-(2-Nitrophenyl) pyrimidine-4-yl)-3-(2-Nitrophenyl imino) indolin-2-one; IR (KBr.cm^{-1}): 1330(Ar-NO_2), 1630(C=N), 3412 (NH2), 1245(C-N) 896(CH=CH of aroma). ^{1}H NMR (300 MH_Z, $DMSO-d_6$, TMS, δ ppm): 5.9(s, 2H, NH_2), 6.8-8.0 (m, Ar-H), 2.3-2.5, 3.4-3.6 and 4.7-5.2 (dd, pyrimidine ring.). MS: m/z : 481.11(M^+).

$8a_2$: 1- 2-amino 6-(2 hydroxy phenyl) pyrimidine-4-yl-3-(Nitro phenyl imino) indoline -2-one; IR(KBr.cm^{-1}): 1330(Ar-NO_2), 3581 (Ar-OH),1636(C=N), 1704(C=O), 3412 (NH2), 1245(C-N) 890 (CH=CH of aroma). ^{1}H NMR (300 MH_Z, $DMSO-d_6$, TMS, δ ppm): 5.9 (s, 2H, NH_2), 10.3 (s.1H, OH), 6.8-8.0 (m, Ar-H), 2.3-2.5, 3.4-3.6 and 4.7-5.2 (dd, pyrimidine ring.). MS: m/z; 452.12(M^+).

$8b_2$: 1-(2-amino-6-(2-hydroxyphenyl) pyrimidine-4-yl)-3-(2-Chlorophenyl imino) indolin-2-one;

IR(KBr.cm^{-1}): 3583 (Ar-OH), 1630(C=N), 648(C-Cl),1734(C=O), 3034 (NH_2 Str), 1245(C-N) 880(CH=CH of aroma). ^{1}H NMR (300 MH_Z, DMd_6, TMS, δ ppm): 5.8 (s, 2H, NH_2), 6.89-7.6 (8H,m,2Ar-H), 2.0-2.4, 3.2-3.4 and 4.4-4.9 (dd, pyrimidine ring.). MS: m/z: 441.10 (M^+).

3.0 **Antioxidant Activity:**

The reducing power [9] of the synthetic drug was determined by the method of Oyaizu. Substances which have reduction potential reacts with potassium ferricyanide (Fe^{+3}) to form potassium ferrocyanide (Fe^{+2}) which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm. Accurately weighed 10mg of the synthetic drug in 1ml of distilled water was mixed into the mixture of 2.5ml of 0.2m phosphate buffer (P^H 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was then incubated at 500C for 20 minutes. Following incubation 2.5ml of 10% trichloro acetic acid was added to the mixture which was then centrifuged at 3000rpm for 10minutes. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and FeCl3 (0.5ml, 0.1%). Increase absorbance of the reaction mixture was indicated the increased reducing power.

4.0 Antimicrobial activity:

The synthesized compounds were screened for their antimicrobial properties against gram positive bacteria *Staphylococcous aureus* (ATCC-77) and *Bacillus subtilis* (ATCC-46), the gram negative bacteria *Pseudomonas mirabilis* (ATCC-126), *E.Coli* (ATCC-324), Salmonella typhi (UC-562) and Shigella dysenteriae (ATCC-3) by agar diffusion cup plate method[10].For antibacterial studies incubation was carried out at 26^0C for 48 hours.. Ampicillin was used as standard drug for antibacterial activity. The synthesized drug solutions were prepared at 100µg/ml using DMF (Di Methyl Formamide) as diluting solvent. Inhibition zones were measured and the diameter was calculated in millimetre.

5.0 Results:

The physical characterisation of the synthesized compounds was cited in table-1.The result of the antioxidant activity in percentage (Table-2) and reducing power in the form of bar diagram was given as follows. The zone of inhibition as anti-bacterial activity was also cited in table-3.

Table: 1 Characterization of Synthesized Compounds.

Compound	R	R_1	MP(^{0}C)*	Yield( )	R_f value
$7a_1$	$-NO_2$	$-NO_2$	122	78	0.53
$7a_2$	$-NO_2$	-OH	117	71	0.49
$7a_3$	$-NO_2$	$-OCH_3$	109	69	0.58
$7b_1$	-Cl	$-NO_2$	139	83	0.72
$7b_2$	-Cl	-OH	175	71	0.69
$7b_3$	-Cl	$-OCH_3$	183	76	0.63
$8a_1$	$-NO_2$	$-NO_2$	170	65	0.50
$8a_2$	$-NO_2$	-OH	187	65	0.54
$8a_3$	$-NO_2$	$-OCH_3$	134	70	0.57
$8b_1$	-Cl	$-NO_2$	180	77	0.82
$8b_2$	-Cl	-OH	179	61	0.77
$8b_3$	-Cl	$-OCH_3$	193	58	0.61

*= $\pm 2^0$C

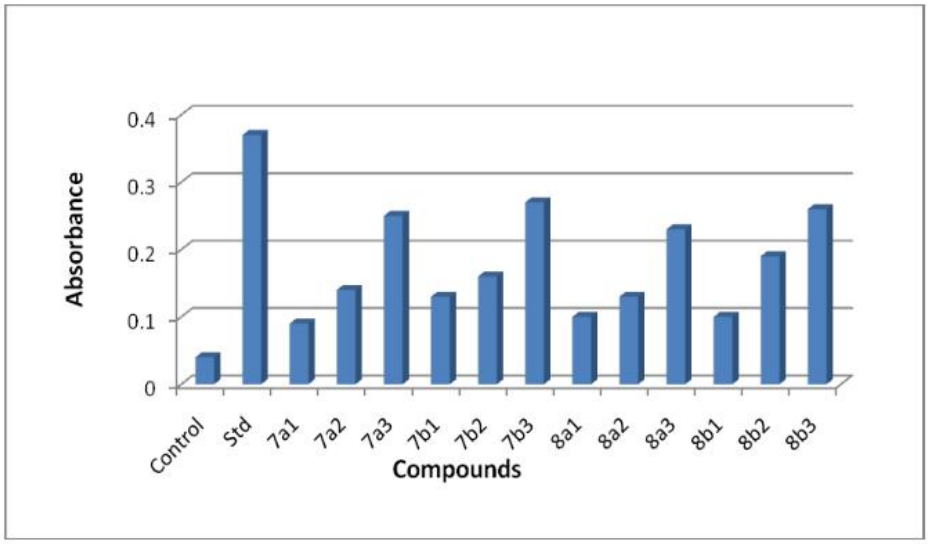

Fig 1: Bar diagram of reducing power activity

Table: 2 Antioxidant activity of the synthesized compounds

Compounds	Antioxidant activity(%)*
Butylated Hydroxy Toleune	89.18
7a1	55.55
7a2	71.42
7a3	84
7b1	69.23
7b2	75
7b3	85.18
8a1	60
8a2	69.23
8a3	82.60
8b1	60
8b2	78.19
8b3	84.61

* Average of three readings.

Table:3 Antibacterial activity of the Synthesized compounds at100µg/ml.

Comp. code	Zone Of Inhibition*(mm) Antibacterial activity					
	Staphylococcus aureus ATCC-77	**Bacillus subtilis ATCC- 46**	**Pseudomonas mirabilis ATCC-126**	**E.Coli ATCC- 324**	**Salmonella typhi UC-562**	**Shigella dysenteriae ATCC-3**
$7a_1$	07	08	12	12	11.3	07
$7a_2$	09	11	12	04	07	12
$7a_3$	14	13	16	12	17	16.3
$7b_1$	06	09	12	13	13.3	11
$7b_2$	11.3	10	09	13	11.2	9.3
$7b_3$	18	14	15	17	14	13.4
$8a_1$	11	09	06	12	8	12.3
$8a_2$	06	09	11.2	08	12	04
$8a_3$	16	17	14	12.3	13	15.3
$8b_1$	09	11	10.3	09	12.2	11.2
$8b_2$	15	12	09	10	13	12
$8b_3$	17	14	17.3	16	14	16.4
Ampicillin	22	20	21	20	23	22
DMF	00	00	00	00	00	00

* Average of three readings.

6.0 Discussion:

The structures of the compounds were elucidated on the basis of elemental analysis. IR, ^{1}H-NMR, and mass spectroscopy. The physical characteristics of the synthesized compounds are present in table-1. Out of 12 compounds synthesized almost all the compounds shows good antioxidant and antibacterial activity. Compounds $7a_2$, $7a_3$, $7b_2$, $7b_3$, $8a_3$ and $8b_3$ shows more promising antioxidant activity in comparison to standard, butylated hydroxy toluene.(Table-2). The compounds also screened for antibacterial activity towards different strains of gram positive Staphylococcus aureus (ATCC-77) and Bacillus subtilis (ATCC-46), the gram negative bacteria Pseudomonas mirabilis (ATCC-126), E.Coli (ATCC-324), Salmonella typhi (UC-562) and Shigella dysenteriae (ATCC-3). Almost all the compounds having very good antibacterial activity. The compounds 7a3, 7b2, $7b_3$, $8a_3$, $8b_2$ and $8b_3$ showed very good zone of inhibition at concentration of 100µg/ml against both gram positive and gram negative bacteria, comparable to standard drug Ampicillin (Table-3).

On critical overview of synthesized compounds, it has been found that compounds with methoxy (-OCH_3) substitution on phenyl ring at para position and hydroxy (-OH) group at ortho position found potent and chloro (-Cl) substitution also makes the compound potent against all bacteria and oxidants. In conclusion a novel series of mercapto-pyrimidine and amino-pyrimidine analogous possessing indole nucleus were synthesized for their potential antioxidant and antibacterial activity. The presence of considerable antioxidant and antibacterial activity in the test compounds may be endorsed to the presence of mercapto- pyrimidines, amino-pyrimidines, indole and phenyl ring, all of which together might have contributed for increase in the reducing power and lipophilic character responsible for penetration of the compound inside the bacterial strain.

7.0 Acknowledgement

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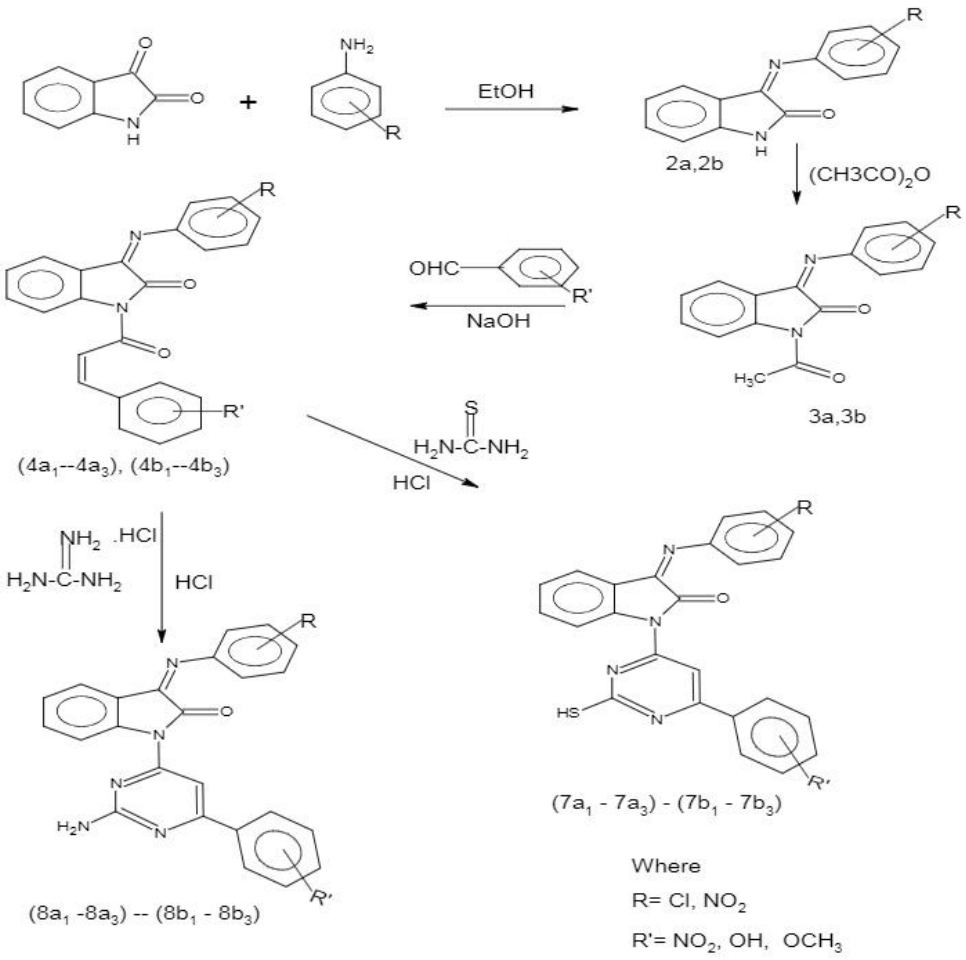
Scheme



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