SYDNONE DERIVATIVES A SYNTHONS FOR NOVEL MESOIONIC COMPOUNDS. SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF SOME 2-(4`-SUBSTITUTED ANILINOSYNDON-3`-YL)-1, 3, 4-THIADIAZINO (6, 5-B) INDOLES

Hemant Panwar[1,*], Shishupal Singh[2], and Nidhi Chaudhary[3]

[1]*Depatment of Chemistry, Neelkanth Institute of Technology, Modipuram-250110, Meerut, U.P., India*

[2]*Department of Chemistry, Aligarh Muslim University, Aligarh-202002, U.P., India*

[3]*Department of Chemistry, I.I.M.T., I.E.T., Ganga Nagar, Meerut-250001, U.P., India*

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ABSTRACT

In the present study, a series of 2-(4´-Substitutedanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7a-j have been synthesized. All the synthesized compounds have been characterized by elemental and spectral (I R, ^{1}H-NMR and Mass) spectrometric analysis. Furthermore, above mentioned compounds were evaluated for their antibacterial and antifungal activities against selected panel of pathogenic strains. Ampicillin trihydrate, ofloxacin and fluconazole, griseofulvin were used as standard drugs for antibacterial and anifungal activity respectively. Compound 7j was found the most potent one with lesser toxicity in the prepared indole derivatives.

Keywords: *Indoles, Antifungal, Antibacterial, Acute toxicity*

INTRODUCTION

Sydnone is a mesoionic heterocyclic aromatic chemical compound. From literature survey, sydnone derivatives are most important member of the mesoionic category of compounds. Sydnone derivatives have been viewed as exotic structures within the heterocyclic community. With few exceptions, sydnones are stable compounds that exhibit significant polarity. Of the many potential applications of sydnones, the one that has attracted the most interest is their biological properties like anticonvulsant [1], antitumor [2], diuretic [3], antimicrobial [4-5], hypotensive [6], anticancer [7] and analgesic [8] activities. On the other hand, bulk of literature is available to explore the wide spectrum of biological activities of indole and its analogs as anti-inflammatory [9-13], anticonvulsant [14], antitumor [15], antimicrobial [16], antibacterial [17-18], antifungal [19]. Hence all these observation encouraged us to synthesize some novel derivatives based on substituted sydnonyl-1, 2, 3-thiadiazino (6, 5-b)indoles.

EXPERIMENTAL SECTION

Materials

All the chemicals used for the preparation of desired derivatives, were obtained from Sisco Research Laboratories (SRL), Mumbai, India; Qualigen Fine Chemicals, Mumbai, India; E. Merck Ltd., New Delhi, India. The reference drugs Ampicillin trihydrate, ofloxacin and fluconazole, griseofulvin were procured from Ind-Swift, Pharmaceutical, Punjab, India and Dr. Redyy Lab., Hyderabad, India.

Instrumentation

The melting points of the compounds were determined in open glass capillaries with the help of thermonic melting points apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. The homogeneity of all the newly synthesized compounds was routinely checked by TLC on silica gel G plates and spots were located by using iodine chamber. Elemental analysis was performed in Heraeus CHN rapid analyzer. The results were found within the ±0.4% of theoretical values. Infrared spectra were recorded on KBr pellets on a Perkin Elmer system 2000 FTIR spectrometer and ^{1}H- NMR spectra on Bruker DPX 200 using TMS as internal standard.

Characterization of the synthesized compounds

The formation of compound 3 was confirmed by the appearance of absorption band at 1712 cm^{-1} due to C=O vibration of COOH group and 3340 cm^{-1} due to NH group which was also evidenced by presence of signals at δ 9.54 ppm and 7.10 due to COOH and NH group, respectively. Disappearance of signal for NH group in ^{1}H NMR spectra and presence of absorption band at 1568 cm^{-1} for N=O group clears the formation of compound 4. Formation of compound 5 was

* Corresponding author. Tel/Fax : +91-121-2578204
Email address : dr_h.panwar@yahoo.co.in

evidenced by absorption band at 841 N-O of sydnones, 1092 C-O of sydnone. Furthermore presence of signal at δ 4.69 ppm due to 1H of sydnone ring cleared the cyclisation of compound 5. In ^{1}H NMR, disappearance of signal at δ 4.69 ppm due to 1H of sydnone ring exhibited the bromination of compound 5 to furnish compound 6 which was also evidenced by disappearance of signal of CH of sydnone ring at 3106 cm^{-1} and by the appearance of absorption band at 600 cm^{-1} due to C-Br group in I. R. spectra. Anilation of sydnone derivatives of indole was confirmed by the presence of broad signal at δ 5.56 of H of NH-Ar group. Furthermore disappearance of absorption band at 600 cm^{-1} due to C-Br group in I. R. spectra, also marked the formation of compound 7a-j.

Procedure

Synthesis of 3-Thiosemicarbazido indole-2-one 1

A mixture of indole-2, 3-dione (0.01 mol), thiosemicarbazide (0.01 mol) in methanol was refluxed for 1 h. The completion of reaction was checked by TLC and excess of methanol was distilled off. The cooled reaction mixture was poured into ice-water, filtered, washed with water, dried and recrystallized from methanol to obtain compound 1: Yield 82%, m.p. 204 °C. IR (KBr) ν_{max} in cm^{-1}: 3420 (NH_2), 3140 (C-H aromatic), 1715 (C=O), 1665 (C=N), 1615 (C=C of aromatic ring), 1205 (C=S).^{1}H-NMR ($CDCl_3$) δ: 9.42 (bs, 1H, NH indolic), 8.90 (bs, 2H, NH_2), 7.75 (bs, 1H, NH), 6.71-7.22 (m, 4H, ArH). MS: $[M]^+$ at m/z 220. Elemental analysis ($C_9H_8N_4SO$), calcd: C 49.09, H 3.63, N 25.45%, found: 49.10, H 3.65, N 25.42%.

Synthesis of 2-Amino-1, 3, 4-thiadiazino (6, 5-b)indole 2

Compound 1 (0.01 mol) was mixed with cold and conc. H_2SO_4 (0.04 mol). The reaction mixture was left at room temperature for 16 h. The reaction mixture was poured into ice-cold water, neutralized with liquid ammonia to obtain solid mass, which was filtered, washed with water, dried and recrystallized from methanol to yield compound 2: Yield 80%, m.p. 230 °C. IR (KBr) ν_{max} in cm^{-1}: 3415 (NH_2), 3138 (C-H aromatic), 1620 (C... C of aromatic ring), 1670 (C=N), 1290 (N-N), 688 (C-S-C). ^{1}H-NMR ($CDCl_3$) δ: 8.55 (bs, 2H, NH_2), 6.60-7.12 (m, 4H, ArH). MS: $[M]^+$ at m/z 202. Elemental analysis ($C_9H_6N_4S$), calcd: C 53.46, H 2.97, N 27.72%, found: 53.50, H 2.95, N 27.70%.

Synthesis of 2-(1, 3, 4-thiadiazino (6, 5-b)indole-2´-yl)aminoaceticacid 3

A mixture of 2-amino-1, 3, 4-thiadiazino (6, 5-b) indole (0.01 mol), chloroaceticacid (0.01 mol) and anhydrous K_2CO_3 (5.0 gm) in methanol (dry, 80 mL) were refluxed for about 18 h on a water bath. On completion of the reaction, the excess of solvent was distilled off under reduced pressure and the resulting solid mass was poured into ice cold water, filtered, dried. The solid thus separated was recrystallised with methanol to give compound **3**: Yield 70%, m.p.165 °C. IR (KBr) ν_{max} in cm^{-1}: 1546 (C—C of aromatic), 1610 (C=N), 1712 (C=O of COOH), 2842 (CH_2), 3000 (OH of COOH), 3051 (aromatic CH), 3340 (NH). ^{1}H-NMR ($CDCl_3$) δ: 4.65 (d, 2H, CH_2), 6.55-6.80 (m, 4H, ArH), 7.10 (ss, 1H, NH, exchangeable with D_2O), 9.54 (s, 1H, COOH, exchangeable with D_2O). MS: m/z 260 $[M]^+$. Elemental analysis ($C_{11}H_8N_4SO_2$), calcd: C 50.76, H 3.07, N 21.53%, found: C 50.70, H 3.10, N 21.45%.

Synthesis of 2-N-nitroso-N-(1, 3, 4-thiadiazino (6, 5-b)indole-2´-yl)iminoacetic acid 4

To a well stirred mixture of compound 3 (0.01 mol) in 40% hydrochloric acid (0.01 mol) at 0-5 °C, a solution of sodium nitrite (0.01 mol) in water (25 mL) was added drop wise during 30 min. The reaction was allowed to stand overnight. Crude reaction mixture was filtered, washed thoroughly with ice cold water and dried in air. The solid thus obtained was recrystallised with ethanol- water to obtain compound 4: Yield 68%, m.p.160 °C. IR (KBr) ν_{max} in cm^{-1}: 1548 (C—C of aromatic), 1568 (N=O), 1615 (C=N), 1710 (C=O of COOH), 2837 (CH_2), 3010 (OH of COOH), 3047 (aromatic CH). ^{1}H-NMR ($CDCl_3$) δ: 4.69 (d, 2H, CH_2), 6.50-6.80 (m, 4H, ArH), 9.60 (s, 1H, COOH, exchangeable with D_2O). MS: m/z 289 $[M]^+$. Elemental analysis ($C_{11}H_7N_5SO_3$), calcd: C 45.67, H 2.42, N 24.22%, found: C 45.70, H 2.40, N 24.45%.

Synthesis of 2-(Sydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indole 5

Compound 4 was heated with acetic anhydride (1:5 by weight) on a water bath for 3 h. The reaction mixture was poured over crushed ice and recrystallised with ethanol to get compound 5: Yield 62%, m.p.136 °C. IR (KBr) ν_{max} in cm^{-1}: 841(N-O of sydnones), 1092 (C-O of sydnone), 1252 (C-N), 1522 (N-N), 1573 (C—C of aromatic), 1620 (C=N), 1752 (C=O of sydnone), 3033 (aromatic CH), 3106 (sydnone -CH). ^{1}H-NMR ($CDCl_3$) δ: 4.69 (s, 1H, sydnone), 6.62-6.90 (m, 4H, Ar-H). MS: m/z 271 $[M]^+$. Elemental analysis ($C_{11}H_5N_5SO_2$), calcd: C 48.70, H 1.84, N 25.83%, found: C 48.76, H 1.86, N 25.80%.

Synthesis of (4´-Bromosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 6

To a suspension of compound 5 (0.05 mol) in ethanol, sodium bicarbonate (0.24 mol) was added at room temperature. The cloudy solution thus obtained becomes clear on addition of bromine (0.05 mol) in ethanol. The reaction mixture was stirred further for 30

min. On completion of the reaction (checked by TLC), reaction mass diluted with water (250 mL). The solid thus obtained was filtered and recrystallised with isopropyl alcohol-water 6: Yield 59%, m.p.196 °C. IR (KBr) v_{max} in cm^{-1}: 600 (C-Br), 688 (C-S-C), 840 (N-O of sydnones), 1095 (C-O of sydnone), 1250 (C-N), 1520 (N-N), 1576 (C—C of aromatic), 1625 (C=N), 1750 (C=O of sydnone), 3035 (aromatic CH). ^{1}H-NMR ($CDCl_3$) δ: 6.50-6.86 (m, 4H, ArH). MS: m/z 359.9 $[M]^+$. Elemental analysis ($C_{11}H_4N_5SO_2Br$), calcd: C 36.67, H 1.11, N 19.44%, found: C 36.30, H 1.25, N 19.30%.

Synthesis of 2-(4´-Substitutedanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7a-j

The solution of compound 6 (0.02 mol) in methanol was refluxed with different substituted anilines (0.02 mol) for 5-8 h. On completion of the reaction, excess of solvent was distilled off and the residue thus obtained was cooled, poured into ice cold water, triturated with petroleum ether (40-60 °C) and recrystallised with appropriate solvents to furnish the products 7a-j.

2-(4´-anilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7a. Yield 54%, m.p.210 °C. IR (KBr) v_{max} in cm^{-1}: 841 (N-O of sydnone), 1197 (C-O of sydnone), 1251 (C-N), 1522 (N-N), 1573 (C—C of aromatic), 1624 (C=N), 1745 (C=O of sydnone), 3033 (aromatic CH), 3304 (N-H). ^{1}H-NMR ($CDCl_3$) δ: 5.56 (brs, 1H, NH-Ar exchangeable with D_2O), 6.55-7.00 (m, 9H, ArH). MS: m/z 362 $[M]^+$. Elemental analysis ($C_{17}H_{10}N_6SO_2$), calcd: C 56.35, H 2.76, N 23.20%, found: C 56.40, H 2.80, N 23.21%.

2-(4´-o-methylanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7b. Yield 40%, m.p. 173 °C. IR (KBr) v_{max} in cm^{-1}: 840 (N-O of sydnone), 1196 (C-O of sydnone), 1252 (C-N), 1520 (N-N), 1571 (C—C of aromatic), 1624 (C=N), 1747 (C=O of sydnone), 3030 (aromatic CH), 3302 (N-H). ^{1}H-NMR ($CDCl_3$) δ: 1.64 (s, 3H, Ar-CH_3), 5.60 (brs, 1H, NH-Ar exchangeable with D_2O), 6.55-7.05 (m, 8H, ArH). MS: m/z 376 $[M]^+$. Elemental analysis ($C_{18}H_{12}N_6SO_2$), calcd: C 57.44, H 3.19, N 22.34%, found: C 57.40, H 3.10, N 22.21%.

2-(4´-m-methoxyanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7c. Yield 50%, m.p.175 °C. IR (KBr) v_{max} in cm^{-1}: 846 (N-O of sydnone), 1203 (C-O of sydnone), 1249 (C-N), 1522 (N-N), 1575 (C—C of aromatic), 1620 (C=N), 1741 (C=O of sydnone), 3033 (aromatic CH), 3300 (N-H). ^{1}H-NMR ($CDCl_3$) δ: 3.60 (s, 3H, OCH_3), 5.56 (brs, 1H, NH-Ar exchangeable with D_2O), 6.65-7.30 (m, 8H, ArH). MS: m/z 392 $[M]^+$. Elemental analysis ($C_{18}H_{12}N_6SO_3$), calcd: C 57.44, H 3.06, N 21.42%, found: C 57.45, H 3.05, N 21.40%.

2-(4´-p-methoxyanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7d. Yield 45%, m.p. 161 °C. IR (KBr) v_{max} in cm^{-1}: 844 (N-O of sydnone), 1200 (C-O of sydnone), 1251 (C-N), 1524 (N-N), 1572 (C—C of aromatic), 1622 (C=N), 1743 (C=O of sydnone), 3034 (aromatic CH), 3304 (N-H). ^{1}H-NMR ($CDCl_3$) δ: 3.50 (s, 3H, -OCH_3), 5.56 (brs, 1H, NH-Ar exchangeable with D_2O), 6.58-7.20 (m, 8H, ArH). MS: m/z 392 $[M]^+$. Elemental analysis ($C_{18}H_{12}N_6SO_3$), calcd: C 57.44, H 3.06, N 21.42%, found: C 57.45, H 3.05, N 21.40%.

2-(4´-o-aminoanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7e. Yield 42%, m.p.189 °C. IR (KBr) v_{max} in cm^{-1}: 845 (N-O of sydnone), 1202 (C-O of sydnone), 1249 (C-N), 1519 (N-N), 1573 (C—C of aromatic), 1624 (C=N), 1743 (C=O of sydnone), 3033 (aromatic CH), 3301 (N-H). ^{1}H-NMR ($CDCl_3$) δ: 5.61 (brs, 1H, NH-Ar exchangeable with D_2O), 6.10 (brs, 2H,-NH_2), 6.55-7.00 (m, 8H, ArH). MS: m/z 377 $[M]^+$. Elemental analysis ($C_{17}H_{11}N_7SO_2$), calcd: C 54.11, H 2.91, N 25.99%, found: C 54.15, H 3.00, N 26.00%.

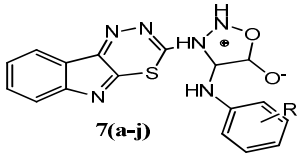
2-(4´-m-aminoanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7f. Yield 44%, m.p.201 °C. IR (KBr) v_{max} in cm^{-1}: 846 (N-O of sydnone), 1199 (C-O of sydnone), 1252 (C-N), 1521 (N-N), 1575 (C—C of aromatic), 1622 (C=N), 1745 (C=O of sydnone), 3031 (aromatic CH), 3307 (N-H). ^{1}H-NMR ($CDCl_3$) δ: 5.50 (brs, 1H, NH-Ar exchangeable with D_2O), 6.15 (brs, 2H,-NH_2), 6.65-7.20 (m, 8H, ArH). MS: m/z 377 $[M]^+$. Elemental analysis ($C_{17}H_{11}N_7SO_2$), calcd: C 54.11, H 2.91, N 25.99%, found: C 54.15, H 3.00, N 26.00%.

2-(4´-p-aminoanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7g. Yield 51%, m.p.176 °C. IR (KBr) v_{max} in cm^{-1}: 840 (N-O of sydnone), 1200 (C-O of sydnone), 1248 (C-N), 1518 (N-N), 1568 (C—C of aromatic), 1626 (C=N), 1740 (C=O of sydnone), 3033 (aromatic CH), 3305 (N-H). ^{1}H-NMR ($CDCl_3$) δ: 5.45 (brs, 1H, NH-Ar exchangeable with D_2O), 6.05 (brs, 2H,-NH_2), 6.60-7.24 (m, 8H, ArH). MS: m/z 377 $[M]^+$. Elemental analysis ($C_{17}H_{11}N_7SO_2$), calcd: C 54.11, H 2.91, N 25.99%, found: C 54.15, H 3.00, N 26.00%.

2-(4´-o-bromoanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7h. Yield 40%, m.p.187 °C. IR (KBr) v_{max} in cm^{-1}: 843 (N-O of sydnone), 1196 (C-O of sydnone), 1250 (C-N), 1524 (N-N), 1575 (C—C of aromatic), 1624 (C=N), 1743 (C=O of sydnone), 3035 (aromatic CH), 3302 (N-H). ^{1}H-NMR ($CDCl_3$) δ: 5.60 (brs, 1H, NH-Ar exchangeable with D_2O), 6.60-7.20 (m, 8H, ArH). MS: m/z 450.9 $[M]^+$. Elemental analysis ($C_{17}H_9N_6SO_2Br$), calcd: C 45.24, H 1.99, N 18.62%, found: 45.15, H 2.02, N 18.60%.

2-(4´-m-bromoanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7i. Yield 45%, m.p.250 °C. IR (KBr) v_{max} in cm^{-1}: 839 (N-O of sydnone), 1199 (C-O of sydnone), 1254 (C-N), 1520 (N-N), 1570 (C—C of aromatic), 1622 (C=N), 1743 (C=O of sydnone), 3031 (aromatic CH), 3306 (N-H). ^{1}H-NMR ($CDCl_3$) δ: 5.56 (brs, 1H, NH-Ar exchangeable with D_2O), 6.55-7.24 (m, 8H, ArH). MS: m/z 450.9 $[M]^+$. Elemental analysis ($C_{17}H_9N_6SO_2Br$), calcd: C 45.24, H 1.99, N 18.62%,

Table 1. Antibacterial and antifungal data for the synthesized compounds 7a-j



7(a-j)

	Antibacterial inhibition (mm)				Antifungal inhibition (mm)			
Comp. no.	***S. aureus***	***E.coli***	***K. pneumoniae***	***P. vulgaris***	***A. fumigatus***	***C. glabrata***	***C. albacans***	***C. krusei***
@ Control	-	-	-	-	-	-	-	-
Ampicillin-trihydrate	16	20	20	20	-	-	-	-
Ofolxacin	18	22	24	22	-	-	-	-
Fluconazole	-	-	-	-	29	25	19	15
Griseofulvin	-	-	-	-	30	32	15	25
7a.	-	5	-	-	-	10	-	8
7b.	-	10	12	15	18	14	-	20
7c.	-	10	10	-	14	12	10	8
7d.	6	8	8	10	12	15	12	12
7e.	-	-	-	12	8	12	14	15
7f.	-	14	10	10	15	14	12	12
7g.	6	-	-	-	12	10	12	14
7h.	-	12	10	8	-	12	14	12
7i.	-	10	12	18	12	12	14	12
7j.	16	18	22	20	24	24	20	15

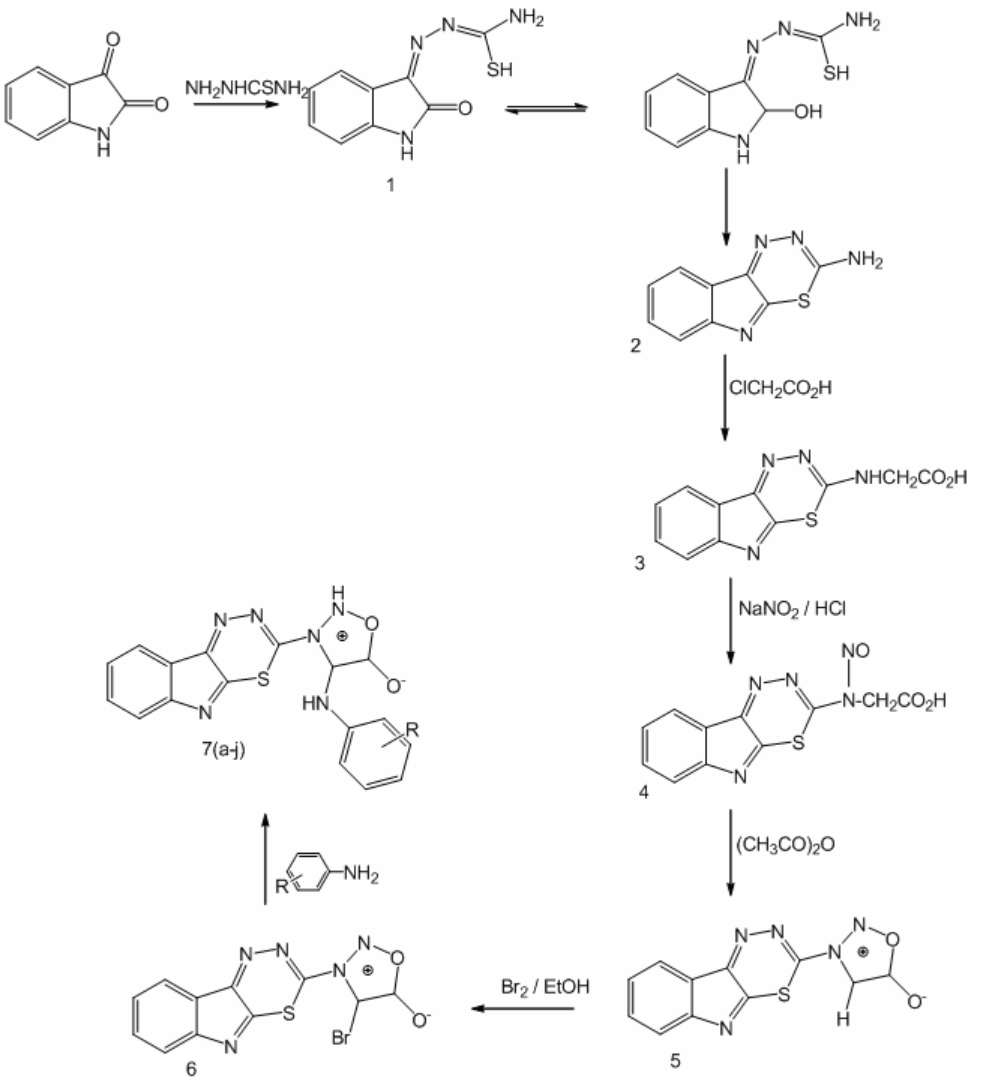
- : **indicates no inhibition**

found: 45.15, H 2.02, N 18.60%.

2-(4´-p-bromoanilinosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7j. Yield 46%, m.p.261 °C. IR (KBr) ν_{max} in cm^{-1}: 840 (N-O of sydnone), 1200 (C-O of sydnone), 1248 (C-N), 1518 (N-N), 1568 (C—C of aromatic), 1626 (C=N), 1740 (C=O of sydnone), 3033 (aromatic CH), 3305 (N-H). ^{1}H-NMR ($CDCl_3$) δ: 5.62 (brs, 1H, NH-Ar exchangeable with D_2O), 6.65-7.30(m, 8H, ArH). MS: m/z 450.9 $[M]^+$. Elemental analysis ($C_{17}H_9N_6SO_2Br$), calcd: C 45.24, H 1.99, N 18.62%, found: 45.15, H 2.02, N 18.60%.

Antimicrobial screening

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. All the bacterial as well as fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. The microorganisms employed antibacterial studies were *Staphylococcus aureus, Escherichia coli, Klabsiella pneumoniae* and *Proteus vulgaris*. Disk diffusion method [20-21] was used for determination of the preliminary antibacterial activity. Disks measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using DMF. One milliliter containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 disks. Disks of each concentration were for placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 °C for 24 h. Ampicillin trihydrate and ofloxacin were used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted. The bacterial inhibition values (mm) of the tested compounds against the tested bacterial strains are recorded in Table 1. On the other hand, the newly prepared compounds were screened for their in vitro antifungal activity against *Aspergillus fumigatus* (plant isolate), *Candida glabrata, Candida albacans* and *Candida krusei* in DMSO by the serial plate dilution method [22-23]. Fluconazole and Griseofulvin were employed as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of the corresponding species. Agar media (20 mL) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch wells were made into each well labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The fungicidal inhibitory (mm) values of the tested compounds against the tested fungal species are recorded in Table 1.



R = C_6H_5, 3-OCH_3.C_6H_4, 4-OCH_3.C_6H_4, 2-NH_2.C_6H_4, 3-NH_2.C_6H_4, 4-NH_2.C_6H_4,2-Br.C_6H_4, 3-Br.C_6H_4,4-Br.C_6H_4

Scheme 1

RESULT AND DISCUSSION

Chemistry

The synthetic work is outlined in scheme-1. 3-Thiosemicarbazido indole-2-one 1 and 2-amino-1, 3, 4-thiadiazino (6, 5-b) indole 2 was prepared by our earlier reported work [24]. The reaction of chloroacetic acid with 2-amino-1, 3, 4-thiadiazino (6,5-b) indole 2 yielded 2-(1, 3, 4-thiadiazino (6,5-b)indole-2´-yl)aminoacetic acid 3 which on further reaction with sodium nitrite and hydrochloric acid furnished 2-N-nitroso-N-(1,3,4-thiadiazino(6,5-b)indole-2´-yl)iminoacetic acid 4. Compound 4 on heating with acetic anhydride gave 2-(sydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indole 5. Reaction of compound 5 with bromine yielded (4´-Bromosydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 6. The title compounds; 2-(4´-Substitutedarylsydnon-3´-yl)-1, 3, 4-thiadiazino (6, 5-b) indoles 7a-j, were prepared by the reaction of compound 6 with different anilines. The structure of these derived congeners were confirmed by spectral (I.R., ^{1}H-NMR and Mass) and elemental (C, H, N) analysis.

Antimicrobial studies

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. For antibacterial studies microorganisms employed were *S. aureus, E.coil, K. pneumoniae and P. vulgaris.*

For antifungal, *A. fumigatus, C. glabrata, C. albacans* and *C. krusei* were used as microorganisms. Both antimicrobial studies were assessed by disk diffusion and serial plate dilution method. The data are summarized in Table 1, and show that all compounds display mild to moderate activity against the tested microorganisms. From SAR we can see that the antibacterial and antifungal activity of the synthesized compounds may be due the presence of the versatile pharmacophore which might increase the lipophilic character of the molecules, which facilitate the crossing through the biological membrane of the microorganism and thereby inhibit their growth.

Acute toxicity study

Lethal dose (LD_{50}) of most potent test compound was determined by the method of Smith [25] in albino mice. After 24 h of drug administration, percent mortality in each group was observed from the data obtained LD_{50}. Data revealed that compound 7j does not show any toxicity up to dose of 9.05 mg/mL body weight in mice.

CONCLUSION

Our present investigation is centered on the studies of reactions, synthesis, spectral analysis and their biological activities. Study of screening data revealed that in compounds 7a-j, halo substituted anilino derivative i.e. 7h–j are biologically more active. Compounds 7h-j, were found to show significant antibacterial as well as antifungal activity and among these compounds, compound 7j was found the most bio-active derivative. On the basis of above result, it was found that –I effect played dominant role in enhancement of biological potency.

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