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DESIGN AND SYNTHESIS OF NEWER SCHIFF BASE DERIVATIVES INTEGRATING SYDNONES AND BENZO[D]THIAZOLE AS POTENTIAL ANTIMICROBIAL AGENTS

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ABSTRACT

Sydnones are very important members of the class mesoionic compounds. A series of schiff base derivatives has been synthesized by condensing 4-(((4-aminophenyl) amino) methyl)-3-(3-nitrophenyl)sydnone with KCNS and Br_2 in presence of glacial HAc^- followed by the reaction with substituted aromatic aldehydes. The synthesized compounds were characterised and evaluated for antimicrobial activities. The newly synthesized compounds were screened against representatives of Gram-positive and Gram-negative bacteria and fungi. Compounds **7b** and **7i** found active against Gram-positive and compounds **7f** and **7j** found active against Gram-negative bacterial strain and compound **7h** found active against fungal strain. All the synthesized compounds were characterized by elemental analysis, FT-IR, ^{1}H-NMR spectra and ^{13}C-NMR.

INTRODUCTION

Drug discovery increasingly requires a common understanding by researchers of the many and diverse factors that go into the making of new medicines. The scientist entering the field will immediately face important issues for which his education may not have prepared him: project teams, patent law, consultants, target product profiles, industry trends, Gantt charts, target validation, pharmacokinetics, proteomics, phenotype assays, biomarkers and many other unfamiliar topics for which a basic understanding must somehow be obtained (Rydzewski, 2008). Heterocyclic compounds are as logical as that of aliphatic or aromatic compounds. Their study is of great interest both from the theoretical as well as practical stand point. Heterocyclic compounds are very widely distributed in nature and are essential to life. They play vital role in the metabolism of all living cells. The genetic material of DNA, the essential amino acids praline, histidine and tryptophan, the vitamins and co-enzyme precursors thiamine, riboflavin, pyridoxine, folic acid and biotin, the B_{12} and E families of vitamins the photosynthesizing pigment chlorophyll, the oxygen transporting pigment haemoglobin and its breakdown products, the bile pigments, the hormones, heteroauxin, serotonin and histamine together with most of the sugars (Potts, 1978). The five membered heterocycles in which an exocyclic heteroatom is attached covalently to the heterocyclic ring through a cyclic heteroatom. This class of compounds known as mesoionic compounds and sydnones are belonging to same class (Wang, 2023; Gupta et al., 2005). Their biological properties (Guhring et al., 2002) which include antibacterial (Savant et al., 2017), antitumor (Davis et al., 1959), antifungal (Savant et al., 2022), antimalarial (Nyberg and Cheng, 1965), antioxidant (Shih and Ke, 2004), analgesic (Satyanarayana and Rao, 1995), anti-inflammatory (Ray and Wagner, 1977). A hydrogen atom at the 4th position of the sydnone ring allows substitution with a wide variety of electrophiles, such as bromination, nitration, acylation, and sulfonation. It seems to be possible to substitute the 4th position by electron-releasing groups such as the methylene group by Mannich reaction (Messmary et al., 2010; Savaliya et al., 2013).

Benzo[d]thiazoles rarely occur as natural products. They form part of the structure of firefly luciferin and are also known as aroma constituents of tealeaves and cranberries or flavour compounds produced by the fungiA. clavatus and P. frondosus. Being a heterocyclic compound, benzo[d]thiazolederivatives find use in various branches of chemical research e.g. in polymerchemistry (Wang et al., 2011), dyes (Volkova et al., 2008), drugs (Kini et al., 2007) etc. Benzo[*d*]thiazoliumsalts have been used in silver photography, essentially as sensitizing dyes.

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A large number of Schiff bases have been found to exhibit pharmacological activity like antibacterial (Savant et al., 2017; Savant et al., 2022), antifungal (Savant et al., 2017; Savant et al., 2022), tuberculostatic (Mohanambal and Arul, 2014), anticancer (Desai and Naik, 2004), anti-inflammatory (Chandra et al., 2014), cardiovascular agents (Demirbas et al., 2004) and antiHIV (Al-Abed et al., 2002).

This much wide application of Sydnones, Mannich base, Benzo[d]thiazole andSchiff base made me to integrate them in one molecule to exhibit new characteristics and applications.

MATERIALS AND METHODS

Experimental

All the chemicals used were of analytical grade and the solvents were distilled before use. All the melting points reported are uncorrected and were recorded using an electro-thermal melting point apparatus. The structure of synthesized compounds was confirmed by elemental analysis (C, H, N) which was performed on Thermo Scientific FLASH 2000 at G.N.F.C. (Gujarat Narmada Valley Fertilizer Company Ltd., Bharuch). Infrared spectra were recorded with FT-IR Spectrophotometer Perkin Elmer in the frequency range 4,000–400 cm^{-1} with samples embedded in KBr disks. Proton nuclear magnetic resonance (^{1}H NMR) spectra of the compound were recorded with a Bruker Advance II 400 *Hz* NMR and carbon (^{13}C) NMR spectra of the compounds were recorded with a Bruker Advance II 400 NMR spectrometer using DMSO-$d_6$as a solvent and tetramethylsilane (TMS) as an internal reference at Sophisticated Analytical Instrument Facilities (SAIF), Chandigarh. Thin-layer chromatography analysis were performed using aluminium backed Silica-gel plates (Merck 60 F524) and examined under short wave ultraviolet (UV) light.

A general procedure for the synthesis of the mentioned Schiff base derivatives[5,7]

Synthesis of (3-nitrophenyl)glycine (2)

This step, a condensation, involved neutralizing an aqueous solution of chloroacetic acid (0.94 g, 0.01 mole) with an equimolar equivalent of 10% NaOH and adding this solution to an aqueous solution of 3-nitro aniline (1.38 g, 0.01 mole) over a period of 4 h. This reaction mixture was heated for 10 h and the clear liquor was then filtered while hot to remove any decomposition product and refrigerated overnight. The resulting crystals were again filtered to obtain compound **2**. Yield 87 %, m.p. 145–147 ºC.

Synthesis of N-(3-nitrophenyl)-N-nitrosoglycine (3)

To an ice-cooled solution of **2** (1.96 g, 0.01 mole) in 40 ml of water, a solution of sodium nitrite (0.69 g, 0.01 mole) in 5 ml of water was added drop by drop with stirring. After stirring for another 2 h and leaving the solution to stand overnight, the reaction mixture was filtered through a Buckner funnel, and the nitro so compound was precipitated by adding concentrated hydrochloric acid to the filtrate. Yellowish needles were obtained as product, yield 87 %, m.p. 154–157 ºC.

Synthesis of 3-(3-nitrophenyl)sydnone (4)

A mixture of **3** (2.835 g, 0.0126 mole) and acetic anhydride (15 ml) was stirred at room temperature for 12 h in the dark. The solution was poured slowly into cold water which was very well stirred. The pH of the content was adjusted to 7.0 with 10 % Sodium bicarbonate solution. The crude sydnone obtained was washed well with water, dried and recrystallized from 95 % ethanol afforded a yield of 92 % of light yellow needles, m.p. 147-149 ºC.

Synthesis of 4-(((4-aminophenyl)amino)methyl)-3-(3-nitrophenyl)sydnone (5)

The mixture of compound 3-(3-nitrophenyl)sydnone (2.07 g, 0.01 mole), paraformaldehyde (0.25 g, 0.00833 mole) and *p*-phenylenediamine (1.296g, 0.012 mole) were added to 10 ml of acetic acid and 10 ml ethanol and whole mixture was heated at 70 ºC for 3 h. After cooling ethanol was distilled off, 20 ml of water was added and neutralized with aqueous sodium bicarbonate to afford the crude product. Recrystallization from 95 % ethanol yielded 96 % of title compound as crystalline solid.m.p. 207-209 ºC.

Synthesis of 4-(((2-aminobenzo[d]thiazol-6-yl)amino) methyl)-3-(3-nitrophenyl)sydnone (6)

The solution of potassium thiocyanate (7.77 g, 0.08 mole) and 4-(((4-aminophenyl)amino)methyl)-3-(3-nitrophenyl)sydnone (3.29 g, 0.01 mole) in glacial acetic acid (20 mL) was cooled and stirred. To this solution bromine (1.6 mL, 0.01 mole) in glacial acetic acid (6 mL) was added drop wise at a rate such that the temperature would not increase above 10 ºC. After the addition has been completed, the solution was additionally stirred for 2 hat 0 ºC and allowed to stand overnight. Water (6 mL) was added to this mixture and refluxed at 85 ºC on sand bath for 3 h and then filtered while hot. The orange residue was placed again in a reaction flask and treated with 10 mL of glacial acetic acid, heated again to 85 ºC for 2 h and filtered the hot solution. The combined filtrate was cooled and neutralized with concentrate ammonia solution to pH 6 to give dark orange precipitates of title compound. The product was filtered, dried and recrystallized from benzene. Yield 83-85%, m.p. 214-216 ºC.

Synthesis of compounds 7_{a-j}

The title compounds were synthesized by the equimolar reaction between 4-(((2-aminobenzo[*d*]thiazol-6-yl)amino) methyl)-3-(3-nitrophenyl)sydnone and various substituted aromatic aldehydes. Each reactant was dissolved in a minimum amount of methanol, then mixed together and followed by addition of catalytic amount of glacial acetic acid. The solution was refluxed for 8-10 h then cooled to room temperature and poured into ice cold water. The solid product was filtered, dried and recrystallized from ethanol.

According to above procedure, all the Schiff base derivatives incorporated with Mannich base and benzo[*d*]thiazole of Sydnone **7a-j** were synthesized using different substituted aromatic aldehyde. The physical constants of synthesized 7_{a-j} are given in **Table 1**and antimicrobial activity are given in

Table 2.

(7_a) IR: (KBr) ν (cm^{-1}): 3428 (Ar–OH), 3218 (–NH–), 2971 (C-H of $–OCH_3$), 2921, 2853 ($–CH_2–$ of Mannich base), 1727 (>C=O of sydnone), 1666 (-C=N- of Schiff base), 1598 (-C=N- of benzo[*d*]thiazole), 1527 (asym.), 1348 (sym.) (–

NO_2), 1248, 1020 (C–O–C of $–OCH_3$), 690 (–S–C– of benzo[*d*] thiazole); ^{1}H NMR (DMSO- d_6): δ (ppm): 3.84 (s, 3H, $–OCH_3$), 4.33 (s, 2H, $–CH_2–$ of Mannich base), 6.61 (d, 1H, Ar–H), 6.74 (d, 1H, Ar–H), 6.79 (s, 1H, –NH–), 6.93 (d, 1H, Ar–H), 7.12 (s, 1H, Ar–H), 7.32 (t, 1H, Ar–H), 7.45 (s, 1H, Ar–H), 7.63 (d, 1H, Ar–H), 7.87 (s, 1H, Ar–H) 8.23 (d, 1H, Ar–H), 8.27 (s, 1H, Ar–H), 9.97 (s, 1H, –OH); ^{13}C NMR (DMSO- d_6): δ (ppm): 47.01 ($–CH_2–$ of $–CH_2–NH–$), 57.32 (C of $–OCH_3$), 92.27 (C4 of Sydnone), 103.49 (Ar-C), 110.27 (Ar–C), 111.78 (Ar–C), 114.79 (Ar–C), 116.98 (Ar–C), 122.06 (Ar–C), 123.43 (Ar–C), 127.19 (Ar–C of –C–N–), 131.02 (Ar–C), 133.24 (Ar–C), 135.45 (Ar–C), 136.78 (Ar–C), 137.31 (Ar–C), 139.45 (Ar–C of –C–N), 140.24 (C_4–sydnone), 148.34 (Ar–C of–C–N–), 149.78 (Ar–C of –C–O–), 150.56 (Ar–C of –C–O–), 159.89 (Ar–C of Schiff base), 169.23 (>C=O of sydnone), 175.31 (C_2 of 4 benzo[*d*]thiazole).

(7c) IR: (KBr) ν (cm^{-1}): 3431 (Ar–OH), 3320 (–NH–), 2925, 2857 ($–CH_2–$ of Mannich base), 1724 (>C=O of sydnone), 1664 (-C=N- of Schiff base), 1601 (-C=N- of benzo[*d*] thiazole), 1527 (asym.), 1348 (sym.) ($–NO_2$), 737 (–S–C–benzo[*d*]thiazole); ^{1}H NMR (DMSO- d_6): δ (ppm): 4.33 (s, 2H, $–CH_2–$ of Mannich base), 6.61 (d, 1H, Ar–H), 6.74 (d, 1H, Ar–H), 6.79 (s, 1H, –NH–), 6.81 (d, 1H, Ar–H), 6.93 (d, 1H, Ar–H), 7.12 (s, 1H, Ar–H), 7.32 (t, 1H, Ar–H), 7.45 (d, 1H, Ar–H), 7.63 (d, 1H, Ar–H), 7.87 (s, 1H, Ar–H) 8.23 (d, 1H, Ar–H), 8.27 (s, 1H, Ar–H), 9.97 (s, 1H, –OH);^{13}C NMR (DMSO- d_6): δ (ppm): 49.28 ($–CH_2–$ of $–CH_2–NH–$), 98.55 (C4 of Sydnone), 102.73 (Ar-C), 111.66 (Ar–C), 114.32 (Ar–C), 117.57 (Ar–C), 120.42 (Ar–C), 121.27 (Ar–C), 121.92 (Ar–C), 126.81 (Ar–C of –C–N–),133.91 (Ar–C), 135.45 (Ar–C), 136.87 (Ar–C), 137.49 (Ar–C of –C–N), 139.35 (C_4–sydnone), 139.65 (Ar–C of –C–O–), 147.54 (Ar–C of –C–O–), 148.34 (Ar–C of–C–N–), 161.63 (Ar–C of Schiff base), 169.26 (>C=O of sydnone), 175.13 (C_2 of benzo[*d*]thiazole).

RESULT AND DISCUSSION

Science behind the above mentioned work

The multi-component condensation of a primary amine or secondary amine and enolizable carbonyl compound with the aim to synthesized aminomethylated products are referred to as the Mannich Reaction. Followed by the incorporation of Benzo[*d*]thiazole unit which further gives different Schiff base derivatives **(7_{a-j})** by reacting with various substituted aldehydes. We use condensation, nitrosation and cyclodehydration by (Desai and Naik, 2004) steps to synthesize 3-(3-nitrophenyl) sydnone**(4)** which on reaction with paraformaldehyde and *p*-phenylene diamine to give aminomethylated compound **(5) (**Gupta et al., 2005). This on further condensed KCNS in presence of glacial $HAc^-$and Br_2 gives compound **(6)**followed by the reaction with substituted aromatic aldehyde in presence of gla. $HAc^-$to give desired Schiff base derivatives**7_{a-j}** (**Scheme-1**).

Elemental Analysis and Spectral data were used to confirm the structures of synthesized compounds. –S–C– stretching of benzo[*d*]thiazole in compounds **7_{a-j}** was observed between 690 to 750 cm^{-1}. Some additional peaks appear due to substitution in aromatic ring. ^{13}C–NMR spectra showed characteristics signal for the carbonyl carbon of sydnone around 169δ ppm, C_2 of benzo[*d*]thiazole around 175 δppm, Schiff base around160δ ppm and methylene carbon around 49δ ppm.

Antimicrobial activity

Controlling microbial population is essential to prevent the spread of diseases, infection, decomposition, contamination and spoilage caused bythem. This is one of the key objective of my current study. The synthesized compounds were screened for their *in vitro* antibacterial activity against Gram positive bacteria viz., *Staphylococcus aureus, Streptococcus pyogenes,*gram negative bacteria viz., *Escherichia coli* and *Pseudomonas aeruginosa*and were also screened for their*in vitro*antifungal activity against pathogenic yeast, *Candida albicans,*and moulds like *Aspergillus niger*and*Aspergillus clavatus*. I used some standard antibacterial compounds like Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin as reference. The antifungal activity was screened *in vitro* against Antifungal compounds, Nystatin and Griseofulvin, were used as standard. The investigation was carried out by Minimum Inhibitory Concentration (MIC) by the classic method named Broth Dilution Method yields a quantitative results for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in tubes.

Compound **7_b** (R = 3–OCH_3, 2–OH) showed excellent activity against *S. aureus*, **7_i** (R = 4 –Cl) showed excellent activity against *S.pyogenes*, **7_j** (R = 2–F)is most active against both Gram negative bacterial strain viz., *E. coli and P. aeruginosa*, **7_f** (R = 4–OCH_3) is highly active against Gram negative bacterial strain viz., *P. aeruginosa*.All other compounds were showed moderate to good activity and some are inactive against all strains.

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Scheme: 1

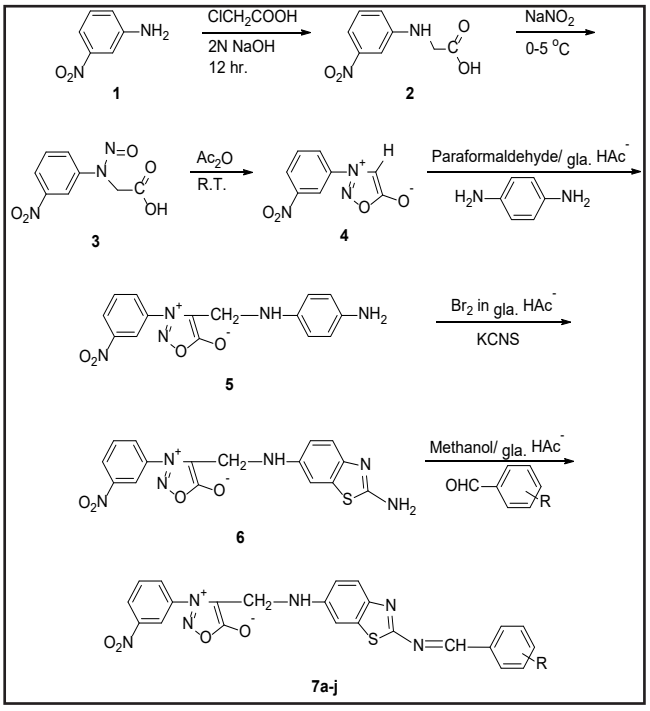

Table 1. Physical constants and Elemental analysis data of compounds 7_{a-j}

No.	R	Yield %	**M.P.** ºC	M. F. & M. W. g/mol	Elemental Analysis		
					% C	% H	% N
7_a	3 –OCH_3, 4 –OH	62	215-217	$C_{24}H_{18}N_6O_6S$ (518.50)	55.32 (55.38)	3.80 (3.87)	16.08 (16.15)
7_b	3 –OCH_3, 2 –OH	72	204-206	$C_{24}H_{18}N_6O_6S$ (518.50)	55.28 (55.38)	3.81 (3.87)	16.15 (16.05)
7_c	2 –OH	73	176-178	$C_{23}H_{16}N_6O_5S$ (488.48)	56.25 (56.32)	3.59 (3.70)	17.05 (17.13)
7_d	4 –NO_2	68	165-167	$C_{23}H_{15}N_7O_6S$ (517.48)	53.08 (53.18)	3.21 (3.30)	18.72 (18.87)
7_e	3,4 –di OCH_3	79	216-218	$C_{25}H_{20}N_6O_6S$ (532.53)	56.11 (56.17)	4.04 (4.15)	15.64 (15.72)
7_f	4 –OCH_3	71	188-190	$C_{24}H_{18}N_6O_5S$ (502.51)	57.10 (57.14)	3.95 (4.00)	16.58 (16.66)
7_g	4 –CH_3	81	191-193	$C_{24}H_{18}N_6O_4S$ (486.51)	58.85 (59.01)	4.08 (4.13)	17.12 (17.20)
7_h	2 –Cl	82	171-173	$C_{23}H_{15}ClN_6O_4S$ (506.92)	54.18 (54.28)	3.28 (3.37)	16.45 (16.51)
7_i	4 –Cl	76	154-156	$C_{23}H_{15}ClN_6O_4S$ (506.92)	54.16 (54.28)	3.25 (3.37)	16.42 (16.51)
7_j	2 –F	72	147-149	$C_{23}H_{15}FN_6O_4S$ (490.47)	56.01 (56.09)	3.28 (3.48)	16.98 (17.06)

Table 2. Antimicrobial activity of Compounds 7_{a-j}

Compounds	**Minimal Inhibition Concentration in mg/ml**						
	Gram –positive		**Gram-negative**		**Fungla strains**		
	S. pyogenes	**S. aureus**	**E. coli**	**P. aeruginosa**	**C. ablicans**	**A. niger**	**A. clavatus**
7_a	200	200	200	200	200	1000	1000
7_b	125	80	250	100	1000	500	1000
7_c	100	100	100	100	>1000	500	500
7_d	250	125	100	125	1000	200	500
7_e	500	125	250	250	1000	500	200
7_f	500	100	200	60	1000	1000	500
7_g	250	200	100	250	500	500	>1000
7_h	200	100	100	200	500	100	250
7_i	60	100	125	100	500	>1000	500
7_j	100	100	60	80	500	250	1000
Gentamycin	0.05	1	0.25	0.5	---	---	---
Ampicillin	100	100	250	100	---	---	---
Chloramphenicol	50	50	50	50	---	---	---
Ciprofloxacin	25	25	50	50	---	---	---
Norfloxacin	10	10	10	10	---	---	---
Nystatin	---	---	---	---	100	100	100
Greseofulvin	---	---	---	---	500	100	100

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