



# World Scientific News

An International Scientific Journal

WSN 142 (2020) 120-134

EISSN 2392-2192

---

---

## Synthesis and biological activity of novel sulfonamides derivatives of various heterocyclic compounds

**Lakshman R. Meena\*, Vinay S. Sharma, Pawan Swarnkar**

Department of Chemistry, Faculty of Basic and Applied Science, Madhav University,  
Madhav Hills N.H.- 27, Opp. Banas River Bridge, P.O. Bharja (Aburoad), Tehsil-Pindwara,  
District- Sirohi (Rajasthan), Pin – 307026, India

\*E-mail address: [laxmanmeena6686@gmail.com](mailto:laxmanmeena6686@gmail.com)

### ABSTRACT

A novel series of Sulfonamide embedded heterocyclic derivatives were synthesized and well characterized by using  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and IR spectroscopic techniques. All the new synthesized compounds were evaluated to their anti-bacterial, anti-fungal and anti-malarial activities. Noticeably, compound 5b was the most potent compound in *vitro* anti-microbial (*S. aureus*) with a MTCC value of 96 microgram/ml and compounds 5g and 5j showed most potent activity in *vitro* anti-fungal (*C. albicans*) with a MTCC value of 277 microgram/ml. Similarly, compound 5e showed high potent activity of anti-malarial (*P. falciparum*) with mean IC<sub>50</sub> value as compared to other synthesized derivatives.

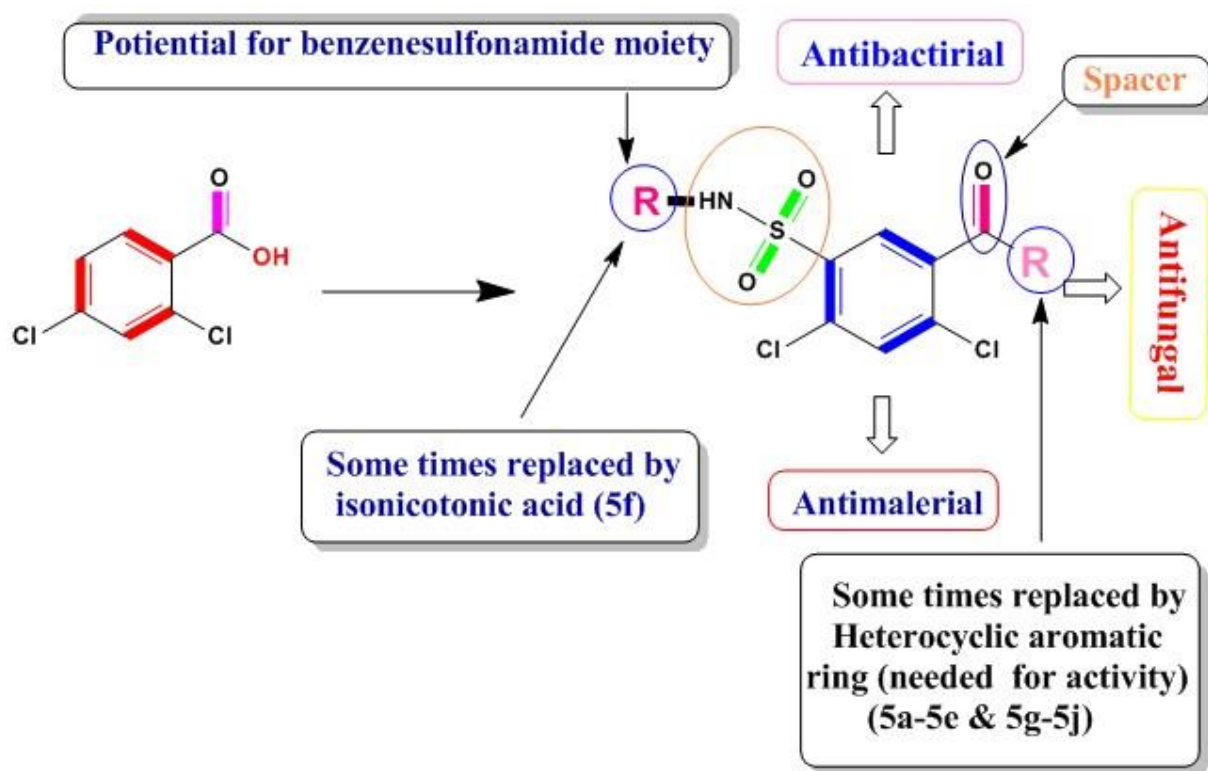
**Keywords:** Sulfonamide, 2,4-dichlorobenzoic acid, anti-microbial, anti-fungal, anti-malarial

### 1. INTRODUCTION

During the past decades, the human population affected with life-treating infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria increased an alarming level around the world. Due to this reason, new classes of antibacterial agents with novel mechanisms are crucial need to combat with the multidrug-resistant

infections. In the past years, some sulphonamide derivatives were developed as new antimicrobial and antifungal agents, are currently used for the treatment of multidrug-resistant Gram-positive infections [1, 2].

Heterocycles containing a sulfonamide compounds possessing a wide spectrum of biological activities such as anti-inflammatory, antiviral and antimicrobial and antitumor properties. Sulfonamide including amino, acid and chloride groups together can be considered as useful intermediates in organic synthesis. Some sulphonamide derivatives were synthesized in our laboratories starting from 2,4-dichloro-5-sulfamoylbenzoic acid. Sulfonamide possess many types of biological activities and representatives of this class of pharmacological agents are widely used in clinic as antibacterial, antifungal, anti-inflammatory [3], antimalarial [4, 5], anti-carbonic anhydrase [6], antitumor [7, 8], antimicrobial, antioxidant [9], antithyroid, diuretic, hypoglycaemic, anti-cancer [10-14], anti HIV [15-19], activities.



**Figure 1.** Geometrical representation of target compounds.

The synthesis and biological activity of *N*-heteroaryl substituted 2,4-dichloro-5-sulfamoylbenzoic acid (5a–5j) were successfully synthesized using the starting material of 2,4-dichlorobenzoic acid. The target molecules (5a–5j) showed good to excellent yield. These targeted sulfonamides were synthesized in good yields through a multi-step protocol and their structures were confirmed by IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and elemental analysis. *In vitro* screening results showed that most of the synthesized compounds showed good inhibitory potentials against Gram positive and Gram negative bacteria in addition with good antimalarial properties as well. The compounds were screened for antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenus* and antifungal activity against *C. albicans*, *A. niger* and *A. clavatus*

and antibacterial activity against *P. falciparum*. The results of the antimicrobial activity presented which enhanced the biological activity against some tested organisms. All newly synthesized compounds were screened for their antimicrobial activity. The antimicrobial activity study revealed that all the compounds displayed good or moderate activity except other synthesized compounds. Those reported variable and numerous biological and pharmacological activities encouraged us to design and synthesize lead molecules of sulphonamide base heterocyclic carrying potential antimicrobial and anti-inflammatory activities. Figure 1 represents the geometrical representation of present target sulphonamide based derivatives and its importance in various applications.

In this research point, we design new compounds based on the biological activity of other heterocycles such as Furan-2-ylmethanamine, Thiophene-2-ylmethanamine, Quinolin-8-ol, 4-(4-aminophenyl)thiazole-2-amine, 4-(2-aminothiazol-4-yl)phenol, 4-(3-aminophenyl)thiazol-2-amine, Isonicotonic acid, 2-amine benzothiazole, 6-methoxybenzothiazol-2-amine, 1,2,4-triazol-4-amine, and 7-hydroxy-4-methyl-2H-chromen-2-one.

### Chemicals

All the chemicals, along with analytical grade solvents, were purchased from Sigma Aldrich, Alfa Aesar (Germany), or Merck through local suppliers. Pre-coated silica gel Al-plates were used for TLC with ethyl acetate and *n*-hexane as solvent system (25:75). Spots were detected by UV254. Gallonkamp apparatus was used to detect melting points in capillary tubes. Melting points were determined on a Buchi B-540 melting point apparatus. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FT IR spectrometer. All the chemicals and heterocyclic derivatives were obtained from Sigma alders and some heterocyclic derivatives were synthesized in laboratory.

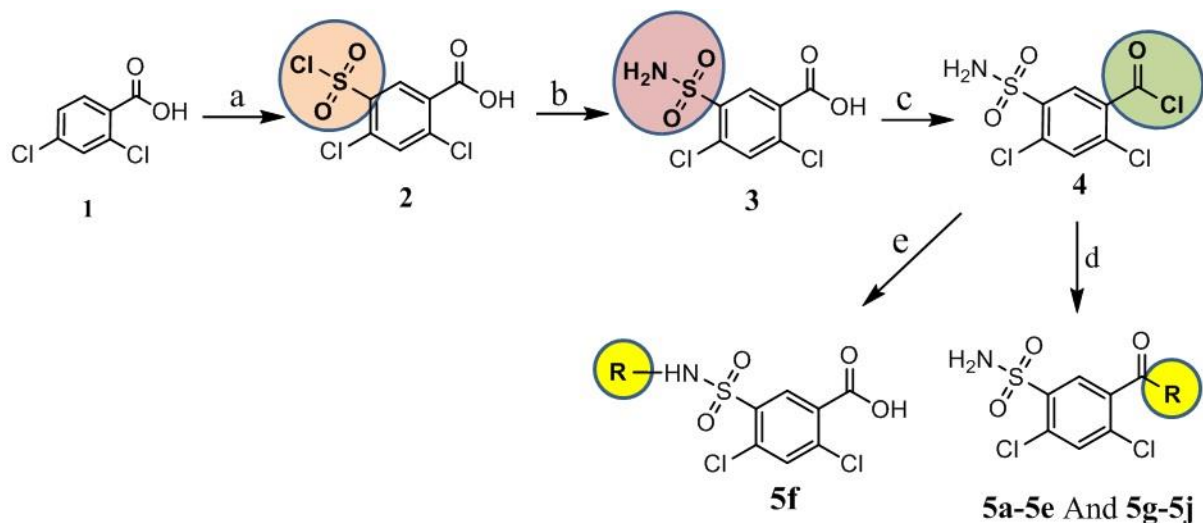
## 2. RESULTS /EXPERIMENTAL

### 2. 1. Experimental Section

#### 2. 1. 1. General Procedures

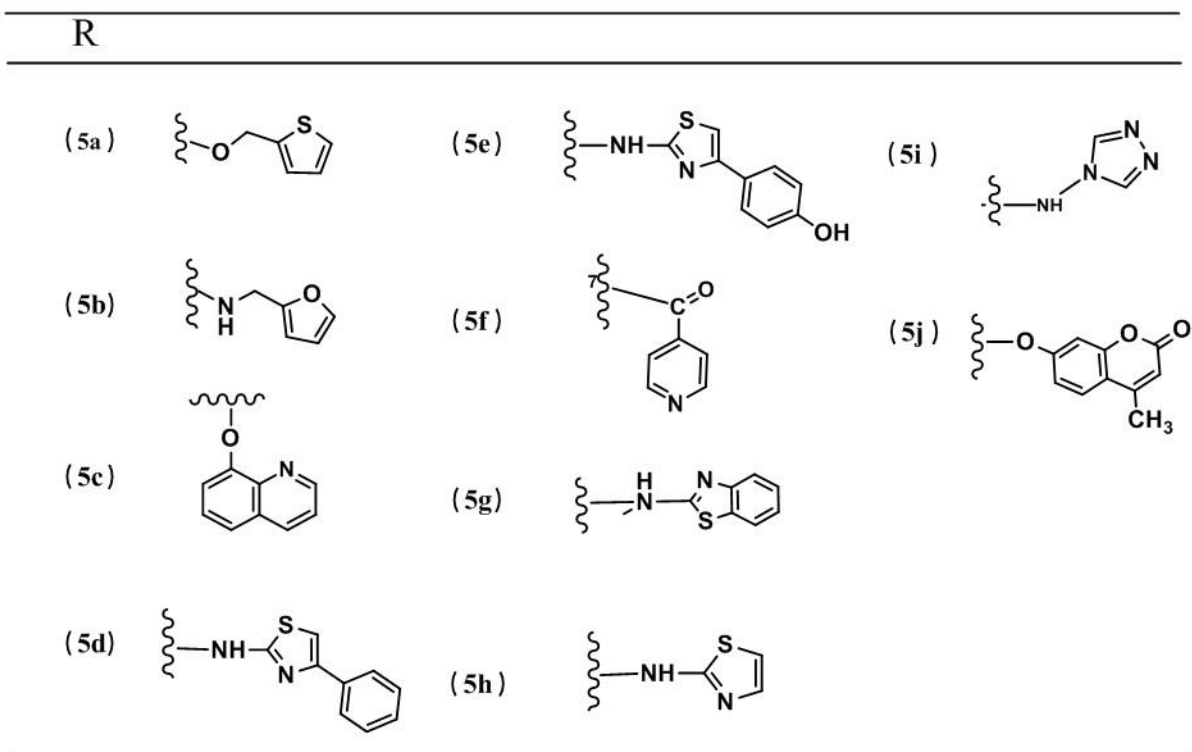
A three-step synthetic strategy was followed for the preparations of sulphonamide based heterocyclic compounds (5a-5j) are mention inabove Scheme 1.

Initially, the reaction of 2-chlorobenzoic acid (1) with chlorosulfonic acid at 0 °C temperature for 2hr to get 2,4-dichloro-5-(chlorosulfonyl)benzoic acid (2). Further, comp. 2 is react with NH<sub>3</sub> at room temperature to produced 2,4-dichloro-5-sulfamoylbenzoic acid (3). Compound (4) obtained by the reaction of (3) with thionyl chloride (SOCl<sub>2</sub>) at 85 °C. In addition, obtained scaffold (4) is further react with different heterocyclic compounds (Table-1) in presence of THF, TEA at 0 °C for 6hr to achieve the target products mention in Scheme-1. The molecular structures of these synthesized derivatives were corroborated by their FT IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectral data along with elemental analysis. The sulphonamide base heterocyclic derivatives (5a-5j) were synthesized from the starting material of 2,4-dichlorobenzoic acid. The synthesis of key intermediate was synthesized by the reaction of 2,4-dichlorobenzoic acid with chlorosulfonic acid.



**Scheme 1. General Synthetic procedure of compounds (5a-5j)**

**Reagents and conditions:** (a) Chlorosulfonic acid, 0°C- 60°C, 3hr (b) NH<sub>3</sub>, TEA, 0°C 2hr (c) Thionyl chloride 85°C, 2hr (d/e) THF, TEA, 0°C-10°C, 6hr.



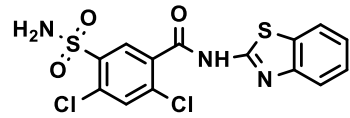
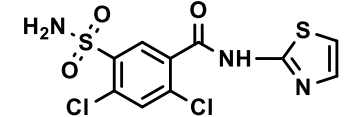
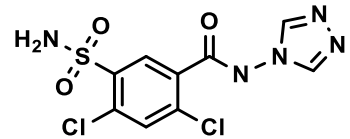
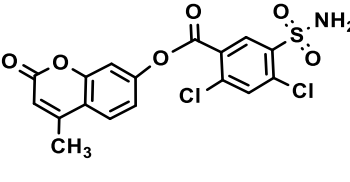
Then treatment of freshly prepared compound 2 with ammonia solution in presence of base TEA produced the 2,4-dichloro-5-sulfamoylbenzoic acid-3. Compound 3 reaction with thionyl chloride to produced 2,4-dichloro-5-sulfamoylbenzoyl chloride (4).

Further treatment of comp. 4 with different amine hydroxyl and acid based hetero derivatives in presence of tetrahydrofuran (THF) and triethylamine (TEA) as base in ice bath

at 0 °C to get the desired product (5a-5j) respectively. The structure of compound 3 was confirmed by IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR spectra. IR spectra of 3 exhibited absorptions at 3000, 3025 cm<sup>-1</sup> for (aromatic C-H stretching), 1700 cm<sup>-1</sup> for (carbonyl group). The <sup>1</sup>H NMR of compound 3 indicates the singlet at δ 10.45 ppm for carboxylic (-COOH) group and aromatic protons resonate as multiplets at δ 7.25-7.92 ppm. The <sup>13</sup>C NMR spectrum of compound 3 showed signals at δ 139.6, 138.1, 137.7, 130.6, 130.2, 125.9 for the carbonyl group and aromatic carbon atom respectively. Similarly, all the target compounds were characterized on the basis of the spectral studies. Further, all the new sulfonamides embedded heterocyclic derivatives (5a–5j) were screened for their *in vitro* anti-bacterial, Anti-fungal and anti-malarial activity using different standard drugs.

**Table 1.** Physical and analytical data of new synthesized compounds (5a-5j).

Comp.	Structure	Mol. Formula	M. W.	%Yield	m. p. (°C)
5a		C <sub>12</sub> H <sub>9</sub> Cl <sub>2</sub> NO <sub>4</sub> S <sub>2</sub>	366.23	66	160°C
5b		C <sub>12</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>4</sub> S	349.18	56	180°C
5c		C <sub>16</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>4</sub> S	397.23	59	185°C
5d		C <sub>16</sub> H <sub>11</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>3</sub> S <sub>2</sub>	428.30	53	110°C
5e		C <sub>16</sub> H <sub>11</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>4</sub> S <sub>2</sub>	444.30	63	80°C
5f		C <sub>13</sub> H <sub>8</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>5</sub> S	375.18	51	130°C

5g		$C_{14}H_9Cl_2N_3O_3S_2$	402.26	67	220°C
5h		$C_{10}H_7Cl_2N_3O_3S_2$	352.20	69	70°C
5i		$C_9H_6Cl_2N_4O_4S$	337.13	61	90°C
5j		$C_{17}H_{11}Cl_2NO_6S$	428.24	64	110°C

## 2. 2. Synthesis of compounds 2, 4-dichloro-5-(chlorosulfonyl)benzoic acid (2)

2,4-dichlorobenzoic acid (0.05 mmol.) was taken in two naked RBF and adds excess amount of chlorosulfonic acid was added drop wise at 0 °C and mixture was refluxed for 2 hours. The reaction progress was observed by TLC using n-hexane and ethyl acetate solvent system (4:6). After completion of reaction, the mixture was allowed to cool at room temperature and reaction mixture was dump in 200 ml crush ice to get white colored precipitates of the product, 2,4-dichloro-5-(chlorosulfonyl)benzoic acid (2). It was filtered and washed with methanol [20].

## 2. 3. Synthesis of compound 2,4-dichloro-5-sulfamoylbenzoic acid (3)

2,4-dichloro-5-(chlorosulfonyl) benzoic acid (0.05 mmol) was taken in RBF and add excess amount of ammonia solution at 0 °C. After it the temperature was maintain between 0 °C to 10 °C for 2 hr. The reaction progress was monitor by TLC using n-hexane and ethylacetate solvent system (4:2). After completion of reaction, the mixture was allowed to cool at room temperature and reaction mixture was dump in 200 ml crush ice to get white colored precipitates of the product, 2,4-dichloro-5-sulfamoylbenzoic acid (3). It was filtered and washed with methanol [20].

## 2. 4. Synthesis of final target compounds (5a-5j)

A mixture of 2 g of 2,4-dichloro-5-sulfamoylbenzoic acid (0.1mmol) and 20 ml of thionyl chloride was allowed to react at 80 °C for 2 hour. After completion of the reaction, the reaction mixture will be cleared and extra thionyl chloride was separated by distillation. In this reaction 2,4-dichloro-5-sulfamoylbenzoyl chloride was formed. Now at 0 °C temperature add THF (10 ml) and amine, hydroxyl and acid derivatives (0.1 mmol) and TEA (1 ml), in this reaction the acid chloride obtained as above was slowly added thereto. The reaction progress was observed by TLC using ethyl acetate and hexane solvent system (2:1). After completion of the

reaction, the formed triethylamine (TEA) hydrochloride was separated by filtration, and silica gel was added to the filtrate. Thus, the product was adsorbed on the silica gel and then purified by column chromatography.

## 2. 5. Spectral characterizations

Spectral characterizations of synthesized compounds 5a–5j are given below:

**Thiophen-2-ylmethyl 2,4-dichloro-5-sulfamoylbenzoate (5a):** White solid powder; yield: 66 %; m.p. 160 °C, IR (KBr):  $\nu_{\max}/\text{cm}^{-1}$ , 3323 (-NH str.), 3087 (C-H str. aromatic), 2851 (C-H str. in -OCH<sub>2</sub> group), 1161, 1393 (-SO<sub>2</sub> of -SO<sub>2</sub>NH<sub>2</sub> group), 1626-1699 (C=O str. of ester group), 1700 (-COO group), 579 (str. of Ar-Cl group). <sup>1</sup>H NMR (500 MHz): 4.12 (s, 2H, -OCH<sub>2</sub>), 7.26 (d, 2H, Ar), 7.59 (s, 1H, Ar), 8.08 (s, 1H, -NH). <sup>13</sup>C NMR: 151.9, 138.3, 135.3, 132.6, 132.3, 77.2, 77.0, 76.7, 50.0, 49.1, 33.8, 32.2, 24.61.

**2,4-dichloro-N-(furan-2-ylmethyl)-5-sulfamoylbenzamide (5b):** Brown solid powder; yield: 56 %; m.p. 180 °C, IR (KBr):  $\nu_{\max}/\text{cm}^{-1}$ , 3352 (-NH str.), 3087 (C-H str. aromatic), 2885 (C-H str. in -OCH<sub>2</sub> group), 1152, 1339 (-SO<sub>2</sub> of -SO<sub>2</sub>NH<sub>2</sub> group), 1621 (C=O str. of ester group), 1703 (-CONH group), 526 (str. of Ar-Cl group). <sup>1</sup>H NMR (500 MHz): 4.14 (s, 2H, -OCH<sub>2</sub>), 7.04 (s, 2H, NH), 7.46-7.53 (s, 2H, Ar), 6.49-7.04 (d, 2H, Ar), 8.39 (s, 1H, -NH). <sup>13</sup>C NMR: 152.9, 138.3, 136.3, 131.3, 76.5, 49.6, 33.8, 32.2, 24.4.

**Quinolin-8-yl 2,4-dichloro-5-sulfamoylbenzoate (5c):** Yellow white solid powder; yield: 59 %; m.p. 185 °C, IR (KBr):  $\nu_{\max}/\text{cm}^{-1}$ , 3323 (-NH str.), 3087 (C-H str. aromatic), 1186, 1362 (-SO<sub>2</sub> of -SO<sub>2</sub>NH<sub>2</sub> group), 1625 (C=O str. of ester group), 1713 (-COO group), 531 (str. of Ar-Cl group). <sup>1</sup>H NMR (500 MHz): 7.26 (s, 1H, Ar), 7.54 (d, 2H, Ar), 8.04 (s, 2H, Ar), 6.49-7.04 (d, 2H, Ar), 4.07 (s, 2H, -NH). <sup>13</sup>C NMR: 160.1, 100.0, 77.3, 77.1, 76.8, 49.2, 34.0, 25.6, 25.0.

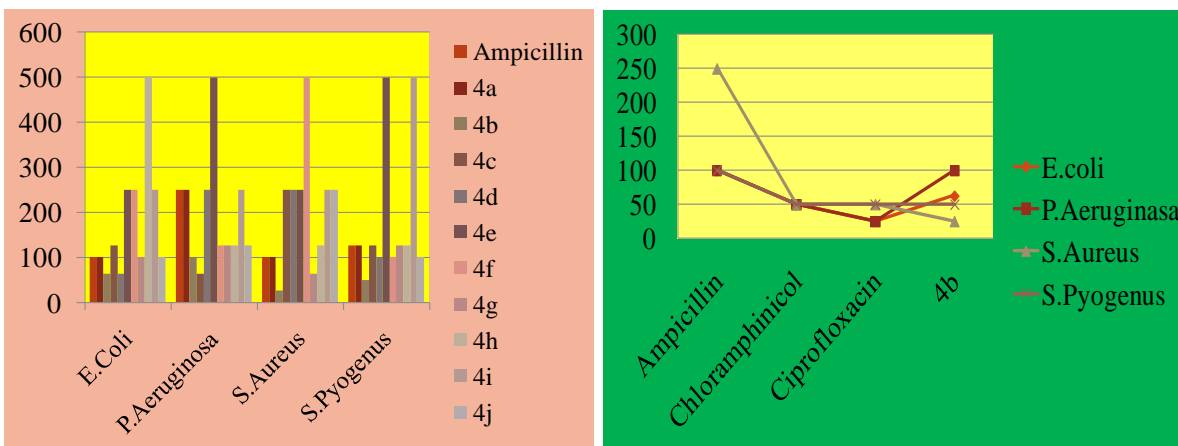
**2,4-dichloro-N-(4-phenylthiazol-2-yl)-5-sulfamoylbenzamide (5d):** White solid powder; yield: 53 %; m.p. 110 °C, IR (KBr):  $\nu_{\max}/\text{cm}^{-1}$ , 3434 (-NH str.), 2850 (C-H str. aromatic), 1165, 1330 (-SO<sub>2</sub> of -SO<sub>2</sub>NH<sub>2</sub> group), 1628 (C=O str. of ester group), 1672 (-CONH group), 548 (str. of Ar-Cl group). <sup>1</sup>H NMR (500 MHz): 5.19 (s, 2H, NH), 6.73 (d, 2H, Ar), 7.74 (s, 1H, Ar), 7.73 (s, 1H, Ar), 7.78 (s, 1H, NH), 7.09 (d, 2H, Ar). <sup>13</sup>C NMR: 167.3, 151.2, 134.5, 128.5, 127.7, 125.9, 102.8, 77.2, 77.0, 76.74.

**2,4-dichloro-N-(4-(4-hydroxyphenyl)thiazol-2-yl)-5-sulfamoylbenzamide (5e):** Brown solid powder; yield: 63 %; m.p. 80 °C, IR (KBr):  $\nu_{\max}/\text{cm}^{-1}$ , 3372 (-OH str.), 3273 (-NH str.), 2958-2859 (C-H str. aromatic), 1152, 1339 (-SO<sub>2</sub> of -SO<sub>2</sub>NH<sub>2</sub> group), 1621 (C=O str. of ester group), 1713 (-CONH group), 1446 (-CH=N), 557 (str. of Ar-Cl group). <sup>1</sup>H NMR (500 MHz): 5.36 (s, 1H, -OH), 7.27 (d, 2H, Ar), 7.66 (s, 2H, Ar), 7.70 (s, 2H, NH), 6.49 (d, 2H, Ar), 8.52 (s, 1H, -NH). <sup>13</sup>C NMR: 163.5, 13.8, 138.1, 134.9, 133.7, 132.3, 128.9, 77.25, 77.0, 76.75, 65.5, 44.3, 29.2, 25.8.

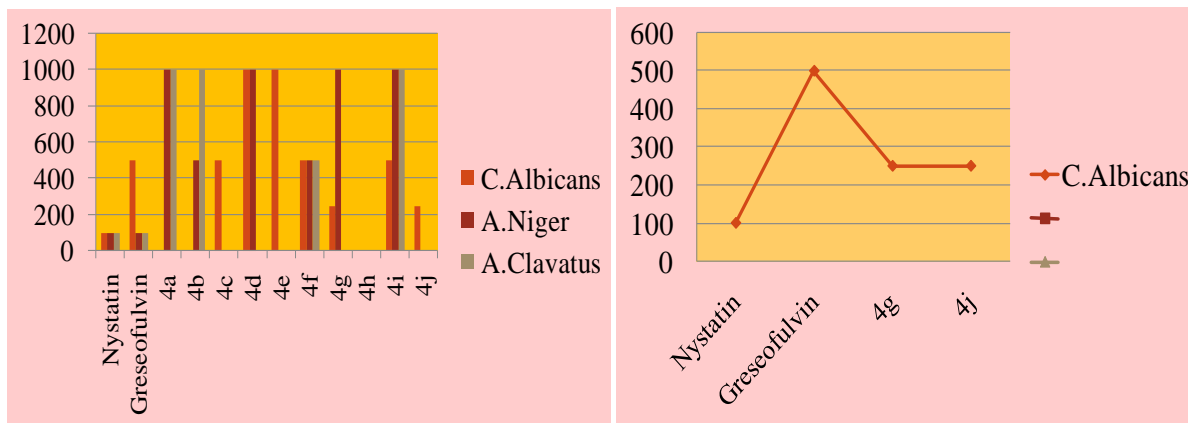
**2,4-dichloro-5-(N-isonicotinoylsulfamoyl) benzoic acid (5f):** White powder; yield: 51 %; m.p. 131 °C, IR (KBr):  $\nu_{\max}/\text{cm}^{-1}$ , 3468 (-OH str. of -COOH group), 3394 (-NH str.), 2927-2852 (C-H str. aromatic), 1152, 1372 (-SO<sub>2</sub> of -SO<sub>2</sub>NH<sub>2</sub> group), 1625 (C=O str. of ester group), 1687 (-CONH group), 1440 (-CH=N), 647, 557 (str. of Ar-Cl group). <sup>1</sup>H NMR (500 MHz):

9.68 (s, 1H, -COOH), 8.67 (s, 1H, -NH), 7.41 (d, 2H, Ar), 7.40 (d, 2H, Ar), 7.28 (s, 1H, Ar), 6.37 (s, 1H, Ar). <sup>13</sup>C NMR: 168.3, 156.8, 153.2, 150.1, 144.2, 120.5, 77.2, 77.0, 76.7, 56.9, 49.8, 49.0, 33.4, 32.6, 30.6, 26.0, 25.5, 24.4.

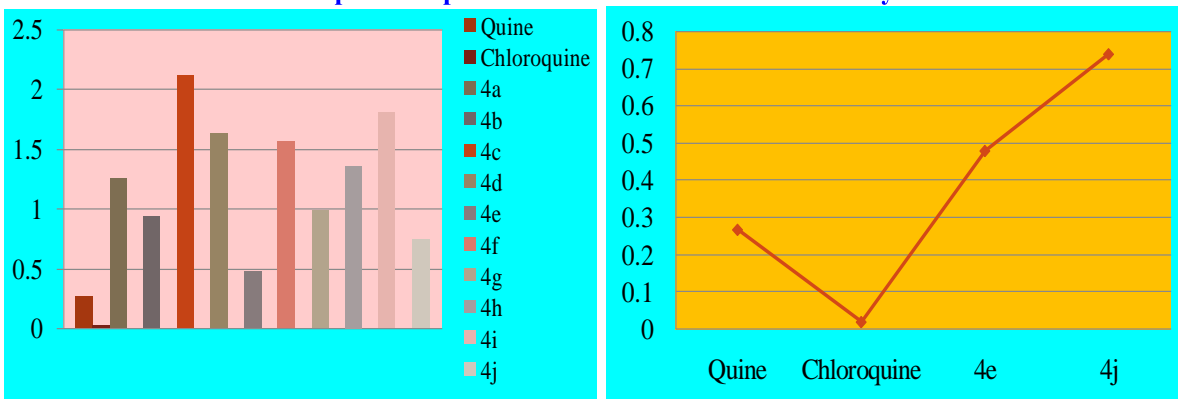
**Graphical representation of Antibacterial activity**



**Graphical representation of Antifungal activity**



**Graphical representation of Antimalarial activity**



**Figure 2.** Graphical representation of representative compounds



**N-(benzo[d]thiazol-2-yl)- 2,4-dichloro-5-sulfamoylbenzamide (5g):** Orange powder; yield: 67 %; m.p. 220 °C, IR (KBr):  $\nu_{\max}/\text{cm}^{-1}$ , 3381 (-NH str.), 2925-2850 (C-H str. aromatic), 1162, 1352 (-SO<sub>2</sub> of -SO<sub>2</sub>NH<sub>2</sub> group), 1683 (C=O str. of ester group), 1731 (-CONH group), 1440 (-CH=N), 647, 568 (str. of Ar-Cl group). <sup>1</sup>H NMR (500 MHz): 8.55 (s, 1H, -NH), 7.66 (s, 2H, Ar), 7.26 (d, 2H, Ar), 5.18 (s, 2H, NH). <sup>13</sup>C NMR: 134.6, 133.6, 132.3, 77.2, 77.0, 76.7, 62.4, 29.6, 14.1.

**2,4-dichloro-5-sulfamoyl-N-(thiazol-2-yl) benzamide (5h):** Brown solid powder; yield: 69%; m.p. 70 °C, IR (KBr):  $\nu_{\max}/\text{cm}^{-1}$ , 3372 (-NH str.), 2958, (C-H str. aromatic), 1152, 1333 (-SO<sub>2</sub> of -SO<sub>2</sub>NH<sub>2</sub> group), 1580 (C=O str. of ester group), 1713 (-CONH group), 1441 (-CH=N), 647, 588 (str. of Ar-Cl group). <sup>1</sup>H NMR (500 MHz): 8.51 (s, 1H, -NH), 7.66 (s, 2H, Ar), 7.27 (s, 2H, Ar), 5.41 (s, 2H, NH). <sup>13</sup>C NMR: 163.5, 138.7, 138.1, 134.9, 133.7, 132.3, 128.8, 77.2, 77.0, 76.7, 65.5, 44.3, 29.0, 25.8.

**4H-1,2,4-triazol-4-2,4-dichloro-5-sulfamoylbenzoate (5i):** Light yellow powder; yield: 61 %; m.p. 90 °C, IR (KBr):  $\nu_{\max}/\text{cm}^{-1}$ , 3557 (-NH str.), 2958-2850 (C-H str. aromatic), 1152, 1336 (-SO<sub>2</sub> of -SO<sub>2</sub>NH<sub>2</sub> group), 1636 (C=O str. of ester group), 1798 (-CONH group), 1453 (-CH=N), 647, 560 (str. of Ar-Cl group). <sup>1</sup>H NMR (500 MHz): 7.26 (s, 2H, Ar), 7.67 (s, 2H, Ar), 5.21 (s, 2H, NH), 8.54 (s, 1H, NH). <sup>13</sup>C NMR: 163.4, 138.8, 138.1, 134.8, 133.7, 132.3, 128.9, 77.2, 77.0, 76.7, 65.5, 44.3, 29.0, 25.9.

**4-methyl-2-oxo-2H-chromen-7yl 2,4-dichloro-5-sulfamoylbenzoate (5j):** White powder; yield: 64 %; m.p. 110 °C, IR (KBr):  $\nu_{\max}/\text{cm}^{-1}$ , 3406 (-NH str.), 3027-2850 (C-H str. aromatic), 1152, 1335 (-SO<sub>2</sub> of -SO<sub>2</sub>NH<sub>2</sub> group), 1627 (C=O str. of ester group), 1773 (-CONH group), 1440 (-CH=N), 559 (str. of Ar-Cl group). <sup>1</sup>H NMR (500 MHz): 2.40 (s, 3H, CH<sub>3</sub>), 7.14 (s, 1H, Ar), 7.27 (s, 1H, Ar), 7.65 (s, 2H, Ar), 8.50 (s, 2H, NH). <sup>13</sup>C NMR: 163.5, 138.7, 138.1, 134.9, 133.7, 132.3, 128.8, 77.2, 77.0, 76.7, 65.5, 44.3, 29.0, 25.8.

## 2. 6. Evaluation of antimicrobial activity

### 2. 6. 1. In vitro antimalarial method

All the synthesized compounds were screened for antimalarial activity in the Microcare laboratory & TRC, Surat, Gujarat. The in vitro antimalarial assay was carried out in 96 well microtitre plates according to the micro assay protocol of Rieckmann and co-workers with minor modifications. The cultures of *P. falciparum* strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200  $\mu\text{l}$  of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O<sup>+</sup>). A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20  $\mu\text{l}$  volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4  $\mu\text{g/ml}$  to 100  $\mu\text{g/ml}$  in duplicate well containing parasitized cell preparation.

The culture plates were incubated at 37 °C in a candle jar. After 36 to 40 h incubation,

thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Quinine and Chloroquine was used as the reference drug.

## 2. 7. Evaluation of Anti-bacterial activity

Novel synthesized sulfonamides heterocyclic derivatives (5a–5j) showed good Anti-microbial activity against representative gram +ve and gram –ve bacteria *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenus* using Broth Dilution Method to evaluate the antibacterial activity. The minimum inhibitory concentration (MIC;  $\mu\text{g ml}^{-1}$ ) was determined for each compound. The MIC is defined as the Minimal Bactericidal Concentration at which complete inhibition of bacterial growth was observed. Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin were used as reference compounds. The MIC values of the synthesized compounds along with the standard drugs for comparison are reported in Table 2.

Among the 10 sulfonamides heterocyclic derivatives (5a–5j) tested, four compounds (5b, 5c, 5d and 5g) were found to be active with MIC values in the range of 25–62.5  $\mu\text{g ml}^{-1}$ . The compound 5b was found to be highly active among all the compounds tested with a MIC value of 25  $\mu\text{g ml}^{-1}$ , which is four times more active than the standard drug Ampicillin (MIC, 100  $\mu\text{g ml}^{-1}$ ) Against *E. coli* bacteria. Compound 5b was found to be equal active among the standard drug Ciprofloxacin with a MIC value is 25  $\mu\text{g ml}^{-1}$  Against *E. coli* bacteria. The antibacterial, antifungal and antimalarial activities of representative compounds were shown in Table 2, Table 3 and Table 4.

**Table 2.** Result of Antibacterial activity of compounds (5a-5j).

Antibacterial activity minimal inhibition concentration [microgram/ml]					
Sr.	Comp.	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenus</i>
No.	No.	MTCC-443	MTCC-1688	MTCC-96	MTCC-442
1	5a	100	250	100	125
2	5b	62.5	100	25	50
3	5c	125	62.5	250	125
4	5d	62.5	250	250	100
5	5e	250	500	250	500
6	5f	250	125	500	100
7	5g	100	125	62.5	125

8	5h	500	125	125	125
9	5i	250	250	250	500
10	5j	100	125	250	100
Standard drug					
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

The preliminary sulfonamide base heterocyclic derivatives analogues reveals that the compounds bearing acid group as well as substituted such as furan-2-ylmethanamine, 8-hydroxy quinoline, 4-phenylthiazol-2-amine, and 2-amine benzothiazole based moiety (5b, 5c, 6d and 5g) do favour better activity. However, the addition of another group like 4-(2-aminothiazol-4-yl)phenol, 2-amine thiazole and 4-amine-1,2,4-triazol leads to complete loss of the activity respectively.

## 2. 8. Evaluation of Anti-fungal activity

**Table 3.** Anti-fungal activity of compounds (5a-5j)

Antifungal activity minimal fungicidal concentration [microgramme/ml]				
Sr. No.	Comp. no.	<i>C. albicans</i>	<i>A. niger</i>	<i>A. clavatus</i>
		MTCC-227	MTCC-282	MTCC-1323
1	5a	>1000	1000	1000
2	5b	>1000	500	1000
3	5c	500	>1000	>1000
4	5d	1000	1000	>1000
5	5e	1000	>1000	>1000
6	5f	500	500	500
7	5g	250	1000	>1000

8	5h	>1000	>1000	>1000
9	5i	500	1000	1000
10	5j	250	>1000	>1000
Standard drug				
Nystatin		100	100	100
Greseofulvin		500	100	100

The anti-fungal potency of the synthesized compounds (5a-5j) was investigated against *C. albicans* (MTCC 227), *A. niger* (MTCC 282) and *A. clavatus* (MTCC 1323). Nystatin and Greseofulvin were used as reference compounds. The MIC values of the synthesized compounds along with the standard drugs for comparison are reported in Table 3. Among the 10 sulfonamides heterocyclic derivatives (5a-5j) tested, two compounds (5g and 5j) were found to be active with MIC values 250  $\mu\text{g ml}^{-1}$ . The compound 5g and 5j were found to be highly active among all the compounds tested with a MIC value of 250  $\mu\text{g ml}^{-1}$ , which is 2 times more active than the standard drug Greseofulvin (MIC, 500  $\mu\text{g ml}^{-1}$ ) against *C. albicans* (MTCC227). While Compounds 5g and 5j were 2.5 times less active than the standard drug Nystatin (MIC, 100  $\mu\text{g ml}^{-1}$ ) against *C. albicans* (MTCC227). Compound 5c, 5f and 5i were found to be equal active among the standard drug Greseofulvin (MIC, 500  $\mu\text{g ml}^{-1}$ ) against *C. albicans* (MTCC227). Compound 5a, 5e and 5h were found to be very less active (MIC, >1000  $\mu\text{g ml}^{-1}$ ) among the standard drug Nystatin (MIC, 100  $\mu\text{g ml}^{-1}$ ) and Greseofulvin (MIC, 500  $\mu\text{g ml}^{-1}$ ) against *C. albicans* (MTCC 227).

## 2. 9. Anti-malarial activity (in vitro)

The anti-fungal activity was tested against the IC50 *P. falciparum*. Quinine and Chloroquine standard drugs were used as reference compounds. The MIC values of the synthesized compounds alongwith the standard drugs for comparison are reported in Table-3. Among all the sulfonamides heterocyclic derivatives (5a-5j) tested, only one compound 5e was found to be highly active with MIC values 0.48  $\mu\text{g/ml}$ , but which is 1.85 times less active than the standard drud of quinine which MIC value (IC 50 0.268)  $\mu\text{g/ml}$ . Compound 5c was minimum active with MIC value is 2.12  $\mu\text{g/ml}$ .

**Table 4.** Anti-malarial activity of compounds (5a-5j)

Sr. No	Compound	Mean IC50 P. Falciparum
1	5a	1.25 $\mu\text{g/ml}$
2	5b	0.93 $\mu\text{g/ml}$
3	5c	2.12 $\mu\text{g/ml}$

4	5d	1.63 µg/ml
5	5e	0.48 µg/ml
6	5f	1.56 µg/ml
7	5g	0.98 µg/ml
8	5h	1.36 µg/ml
9	5i	1.80 µg/ml
10	5j	0.74 µg/ml
Standard drug		
Quinine		IC50 0.020 µg/ml
Chloroquine		IC 50 0.268 µg/ml

### 3. CONCLUSIONS

A series of 2,4-dichloro-5-sulfamoylbenzoic acid derivatives of various Heterocyclic compounds have been synthesized and evaluated for anti-bacterial, anti-fungal activity and anti-malarial activity. The compound 5a and 5g were found to be having moderate antibacterial activity against *E.coli* while 5b and 5d were found good active against *E. coli*. The compound 5b was found to be having moderate activity against *P. aeruginosa* while 5c was found to be more active against *P. aeruginosa*. The activity of compound 5a, 5b and 5g were found good against *S. aureus*. The compound 5d, 5f and 5j were found to be having moderate activity against *S. pyogenus* while 5b was found good active against *S. pyogenus*. In the same way 5g and 5j were shown normal Anti-fungal activity against *C. albicans*. The compound 5e showed moderate antimalarial activity against *P. Falciparum*. So, the results obtained from antibacterial, antifungal and antimalarial activities of synthesized compounds are normal, as the compounds showed significant activity as compared to standard or marketed drugs. These results can be used further to design and develop novel antimicrobial agents.

#### Acknowledgements

The authors are thankful to the Department of chemistry, Madhav University, Pindwara, Sirohi, Rajasthan for providing research facilities and laboratory.

#### References

- [1] J. Drews, Drug Discovery: A Historical Perspective. *Science*, 287(5460) (2000), 1960-1964

- [2] J. Zitko, M. Dolezal et al, Synthesis and antimycobacterial properties of N-substituted 6-amino-5-cyanopyrazine-2-carboxamides. *Bioorganic & Medicinal Chemistry* 19(4) (2011) 1471-1476
- [3] A.P. Keche, G.D. Hatnapure et al A novel pyrimidine derivatives with aryl urea, thiourea and sulfonamide moieties: Synthesis, anti-inflammatory and antimicrobial evaluation. *Bioorg Med. Chem. Lett* 22(10) (2012) 3445-3448
- [4] J.N. Dominguez, C. Leon et al, Synthesis and antimalarial activity of sulfonamide chalcone derivatives. *Farmaco* 60(4) (2005) 307-311
- [5] M. Padmanilayam, B. Scorneaux, et al. Antimalarial activity of N-alkyl amine, carboxamide, sulfonamide, and urea derivatives of a dispiro-1,2,4-trioxolane piperidine. *Bioorg Med Chem Lett* 16(21) (2006) 5542-5545
- [6] T. S. Claudiu, Carbonic Anhydrase Inhibition/Activation: Trip of a Scientist Around the World in the Search of Novel Chemotypes and Drug Targets. *Current Pharmaceutical Design* 16(29) (2010) 3233-3245
- [7] Y. Kwon, J. Song at all, Design, Synthesis, and Biological Activity of Sulfonamide Analogues of Antofine and Cryptopleurine as Potent and Orally Active Antitumor Agents. *J. Med. Chem.* 58(19) (2015) 7749-7762
- [8] El-Sayed NS, El-Bendary ER, El-Ashry SM, El-Kerdawy MM. Synthesis and antitumor activity of new sulfonamide derivatives of thiadiazolo[3,2-a] pyrimidines. *Eur J Med Chem* 46(9) (2011) 3714-20
- [9] H. Ozkana and B. Demirci, J., Synthesis and Antimicrobial and Antioxidant Activities of Sulfonamide Derivatives Containing Tetrazole and Oxadiazole Rings. *Heterocyclic Chemistry* 56(9) (2019) 2528-2535
- [10] C.T. Supuran, A. Casini et al. Protease Inhibitors of the Sulfonamide Type: Anticancer, Antiinflammatory, and Antiviral Agents. *Medicinal Research Reviews* 23(5) (2003) 535-558
- [11] Supuran, C.T. and Scozzafava, A. Carbonic Anhydrase Inhibitors and Their Therapeutic Potential. *Expert Opinionon Therapeutic Patents* 10 (5) (2000) 575-600
- [12] M.M. Ghorab, F.A. Ragab et al. *in Vitro* Anticancer Screening and Radiosensitizing Evaluation of Some New Fluorine-Containing Quinoline and Pyrimidoquinoline Derivatives Bearing a Sulfonamide Moiety. *Medicinal Chemistry Research* 45(9) (2010) 3677-3684
- [13] A.E. Boyd 3rd, Sulfonylurea Receptors, Ion Channels, and Fruit Flies. *Diabetes*, 37(7) (1988) 847-850
- [14] M.M. Kamel, H.I. Ali et al. Synthesis, Antitumor Activity and Molecular Docking Study of Novel Sulfonamide-Schiff's Bases, Thiazolidinones, Benzothiazinones and Their CNucleoside Derivatives. *European Journal of Medicinal Chemistry* 45(2) (2010)572-580
- [15] M. Khanusiy, Z. Gadhawala. Design, Synthesis and Biological Evaluation of Some Novel Chalcone-Sulfonamide Hybrids. *Chemical Science Transactions* 8(2) (2019) 195-207

- [16] R. Vangala, S. K. Sivan, S.R. Peddi, V. Manga, Computational design, synthesis and evaluation of new sulphonamide derivatives targeting HIV-1 gp120. *Journal of Computer - Aided Molecular Design*, 34(1) (2020) 39-54
- [17] M. Zhu, L. Ma et al, Preliminary SAR and biological evaluation of potent HIV-1 protease inhibitors with pyrimidine bases as novel P-2 ligands to enhance activity against DRV-resistant HIV-1 variants. *European Journal of Medicinal Chemistry*, 185(1) (2020) 111866
- [18] P. Selvam, M. Chandramohan et al, Synthesis and anti-HIV activity of 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene) amino]-N(4,6-dimethyl-2-pyrimidinyl)-benzene sulfonamide and its derivatives. *European Journal of Pharmaceutical Sciences* 14(4) (2001) 313-316
- [19] N. A. Al-Masoudi, Y. A. Al-Soud, New Sulphonamide and Carboxamide Derivatives of Acyclic C-Nucleosides of Triazolo-Thiadiazole and the Thiadiazine Analogues. *Synthesis, Anti-HIV, and Antitumor Activities* 27(9) (2008) 1034-1044
- [20] Yagami, Keisuke, Sulfamoylbenzoic acid derivatives and pharmaceutical compositions comprising. (1983). European Patent Office: EP0068239A2, 11.6.82