Microwave assisted synthesis of novel N-(benzo[d] thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)amino)acetamide derivatives as potent antibacterial agents

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ABSTRACT. As part of continuing studies in emerging new antibacterial agent, a novel series of N-(benzo[d] thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3, 2'-thiazolidin]-3'-yl)amino)acetamide derivatives were synthesized by sequencing reaction of 2-aminobenzothiazole with chloroacetyl chloride, hydrazine hydrate, isatin and Thioglycolic acid. The novel heterocycles were characterized by elemental analyses and various spectroscopic techniques. The synthesized compounds were tested in vitro antibacterial activity against two Gram-positive and two Gram-negative bacteria. Bacteriological results showed that compound 5c possessed a broad spectrum of antibacterial activity against the tested microorganisms.

1. INTRODUCTION

Heterocycles compound have one of the prime area of research in the field of organic chemistry. They have donated a developed society from biological and industrial advances. Among them, sulfur and nitrogen-containing heterocyclic compounds have sustained their interest to researchers for historical development in organic synthesis. The grounds of this interest for their biological activities and unique structures that led to several applications in different areas of pharmaceutical and agrochemical research or, more recently in material sciences. Microwave-assisted organic synthesis has rapidly grown in the last decade as an efficient tool for synthetic chemists. Advantage of this methodology such as enhanced rate of reaction, high yields, enhanced purity, and greener conditions make it an attractive technique for applications in the area of drug discovery and drug development research. Considering the benefits of microwave-assisted organic synthesis, synthetic chemist frequently feats this tool in developing synthetic methodologies.[1]

Benzothiazole is one of the accessible heterocyclic compounds, which found in many natural product such as alkaloids. It derived from terrestrial and marine natural products. It is an essential building block in organic synthesis, which serves as a key template for the development of various therapeutic agents. Benzothiazole are an important class of bioactive and industrially important organic compounds. From the current literature, these derivatives are also known to possess antitubercular, cardiovascular, antimicrobial, antiviral, antitumor, anti-microbial, anti-inflammatory, anticonvulsants, anti diabetic, antipsychotic, antidiuretic, anthelmintic, antimalarial, anti-hypertensive, anti-inflammatory, antibacterial, anti-HIV, hypnotic, anti-allergic and analgesic, fibrinogen receptor antagonists with antithrombotic activity, inhibitors of bacterial DNA gyrase B and cytotoxic activities.[2]

Isatin is a privileged class of the heterocyclic compound for synthesizing potential bioactive agents, and its derivatives have been possessed a broad spectrum of bioactivity such as anti-viral,[3] anti-convulsants agents,[4] anti-fungal,[5] anti-tumor,[6] anti-HIV,[7] etc. These interesting properties have encouraged many efforts toward the synthesis and biological screening of isatin derivatives.

Heterocyclic spirooxindoles have produced significant synthetic interest owing to their existence in diverse natural products and distinguished biological activity.[8-13] The sole structural collection and the extremely noticeable pharmacological activity showed that spirooxindole compounds have made them attractive synthetic targets.[14] Spirooxindoles have established more
attention as a result of the wide range of useful pharmacological properties and biological activities, such as antimicrobial, antitumor, antibiotic agents, CRTH2 receptor antagonist and microtubule assembly.

There are very scarce recent literature data on antimicrobial potentials of benzothiazole containing isatin that should combine promising structural properties of not only isatin but also benzothiazole moiety. Therefore, in the present paper, we have prepared a set of new series of N-(benzo[d]thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)amino)acetamide derivative and their in-vitro antibacterial activities against Gram-positive and Gram-negative bacteria. To the best of our knowledge, microwave assisted synthesis of N-(benzo[d]thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl) amino)acetamide derivative have not been documented in the literature.

2. EXPERIMENTAL SECTION

All Starting materials and other reagents were purchased from commercial suppliers. The reactions were monitored by thin layer chromatography (TLC) and terminated as judged by the consumption of starting material. Analytical thin-layer chromatography (TLC) was performed on silica gel G 60 F254 (Merck) plates and eluted with the appropriate solvent ratios (v/v). The melting points were recorded on optimel automated melting point system and were uncorrected. IR spectra was recorded on a Perkin–Elmer 377 spectrophotometer, 1H NMR spectra was measured on Bruker AV 400 MHz using DMSO as a solvent and TMS as an internal standard. Mass spectra were recorded on Advion Expression CMS, USA. Elemental analyses were performed on the vario MICRO cube, elementar CHN analyser serial no.: 15084053.

General procedure of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide derivative (2a-2g):
chloroacetyl chloride (0.01mmol) was added dropwise to a mixture of 2-amino-benzothiazole 1a-1g derivative (0.01 mmol) and triethylamine (0.01 mL) in CHCl3 (20 mL). The reaction mixture was heated under refluxed for 12 h, then the solvent was evaporated under reduced pressure and the solid obtained was recrystallized.[21]

General procedure of N-(benzothiazol-2-yl)-2-hydrazinylacetamide derivative (3a-3g):
Derivatives of N-(benzothiazol-2-yl)-2-hydrazinylacetamide were prepared by refluxing a mixture of compound 2a-2g (0.01 mmol) and hydrazine hydrate 99 % (0.01 mmol) in ethanol (30 mL) for 14 h. The reaction mixture was cooled, and the solid was precipitated out by filtration, washed with water, dried, and recrystallized.

General procedure of (Z)-N-(benzo[d]thiazol-2-yl)-2-(2-(2-oxoindolin-3-ylidene)hydrazinyl) acetamide derivative (4a-4g):
Compounds N-(benzothiazol-2-yl)-2-hydrazinylacetamide derivative (3a-3g) (0.01 mmol) were further treated with different isatin (0.01 mmol) in ethanol in the presence acetic acid as catalyst and 4-5 drops of fused sodium acetate and refluxed for 24 hours. The completion of the reaction was monitored by the TLC. Resulting solid was separated out, filtered, and washed with water, dried and recrystallized by alcohol to get (Z)-N-(benzo[d]thiazol-2-yl)-2-(2-(2-oxoindolin-3-ylidene)hydrazinyl) acetamide derivative (4a-4g).

General procedure of N-(benzo[d]thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3,2'thiazolidin]-3'yl)amino)acetamide derivative (5a-5g):
An equimolar mixture of N-(benzo[d]thiazol-2-yl)-2-(2-(2-oxoindolin-3-ylidene)hydrazinyl) acetamide derivative (4a-4g) (0.01 mmol) and Thioglycolic acid (0.01 mmol) in ethanol was irradiated with microwave for 5-7 min at 350 W in the presence of p-TSA. After the completion of reaction (TLC analysis) using Ethyl acetate: Hexane (30 %) as mobile phase. The reaction mixture was treated with water. The resultant solid was filtered under reduced pressure and the residue washed with methanol gave a pure product (5a-5g) in 81.8-70.2 % of yield.
N-(benzo[d]thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'yl)amino)acetamide (5a): IR (KBr) ($\nu_{\text{max}}$ cm$^{-1}$): 3078 (C-H Aromatic), 3289 (N-H), 1560 (C=O amide); $^1$H NMR (400 MHz, DMSO) $\delta$ 12.04 (s, 1H), 9.79 (s, 1H), 8.19 – 7.78 (m, 2H), 7.46 – 7.25 (m, 4H), 7.23 – 7.08 (m, 2H), 4.08 (s, 1H), 3.56 – 3.44 (m, 3H), 3.03 (s, 1H); ESI-MS: m/z Calculated 425.48, found [M+H]$^+$ 426.5. Anal. Calcd for C$_{15}$H$_{15}$N$_{5}$O$_{3}$S$_{2}$: C, 53.63; H, 3.55; N, 16.46; O, 11.28; S, 15.07 %; found C, 53.33; H, 3.90; N, 16.26; S, 15.18 %.

2-(2-(6-chloroquinolin-4-yl)hydrazinyl)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide (5b): IR (KBr) ($\nu_{\text{max}}$ cm$^{-1}$): 3090 (C-H Aromatic), 3346 (N-H), 1658 (C=O amide), 1656 (C=O amide); $^1$H NMR (400 MHz, DMSO) $\delta$ 12.08 (s, 1H), 9.79 (s, 1H), 7.99 (d, 1H), 7.89 (d, 1H), 7.36 – 7.23 (m, 3H), 7.23 – 7.08 (m, 2H), 4.08 (s, 1H), 3.56 – 3.44 (m, 3H), 3.03 (s, 1H); ESI-MS: m/z Calculated 439.51, found [M+H]$^+$ 440.5. Anal. Calcd for C$_{20}$H$_{17}$N$_{5}$O$_{3}$S$_{2}$: C, 54.65; H, 3.90; N, 15.93; O, 10.92; S, 14.59 %; found C, 54.82; H, 3.60; N, 15.73; S, 14.20 %.

2-(2-(6-chloroquinolin-4-yl)hydrazinyl)-N-(6-fluorobenzo[d]thiazol-2-yl)acetamide (5c): IR (KBr) ($\nu_{\text{max}}$ cm$^{-1}$): 3065 (C-H Aromatic), 3379 (N-H), 1598 (C=O amide), 1678 (C=O amide); $^1$H NMR (400 MHz, DMSO) $\delta$ 11.94 (s, 1H), 9.79 (s, 1H), 7.85 – 7.68 (m, 2H), 7.31 (dd, 2H), 7.23 – 7.08 (m, 3H), 4.08 (s, 1H), 3.56 – 3.43 (m, 3H), 3.05 (s, 1H); ESI-MS: m/z Calculated 443.47, found [M+H]$^+$ 444.4; Anal. Calcd for C$_{19}$H$_{15}$F$_{3}$N$_{5}$O$_{3}$S$_{2}$: C, 51.46; H, 3.18; F, 4.28; N, 15.79; O, 10.82; S, 14.46 %; found C, 51.80; H, 3.26; F, 4.60; N, 15.53; S, 14.86 %.

2-(2-(6-chloroquinolin-4-yl)hydrazinyl)-N-(6-methoxybenzo[d]thiazol-2-yl)acetamide (5d): IR (KBr) ($\nu_{\text{max}}$ cm$^{-1}$): 3046 (C-H Aromatic), 3348 (N-H), 1576 (C=O amide), 1686 (C=O amide); $^1$H NMR (400 MHz, DMSO) $\delta$ 10.96 (s, 1H), 9.83 (s, 1H), 8.01 (d, 1H), 7.91 (d, 1H), 7.37 – 7.22 (m, 3H), 7.20 – 6.04 (m, 2H), 4.96 (s, 1H), 3.79 (s, 3H), 3.58 – 3.45 (m, 4H); ESI-MS: m/z Calculated 455.51, found [M+H]$^+$ 456.5. Anal. Calcd for C$_{20}$H$_{17}$N$_{5}$O$_{4}$S$_{2}$: C, 52.74; H, 3.76; N, 15.37; O, 14.05; S, 14.08 %; found C, 52.96; H, 3.89; N, 15.58; S, 14.28 %.

2-(2-(6-chloroquinolin-4-yl)hydrazinyl)-N-(6-nitrobenzo[d]thiazol-2-yl)acetamide (5e): IR (KBr) ($\nu_{\text{max}}$ cm$^{-1}$): 3063 (C-H Aromatic), 3375 (N-H), 1546 (C=O amide), 1662 (C=O amide); $^1$H NMR (400 MHz, DMSO) $\delta$ 10.19 (s, 1H), 10.01 (s, 1H), 8.89 (d, 1H), 8.10 (dt, 2H), 7.45 – 5.38 (m, 4H), 5.08 (s, 1H), 3.53 – 3.38 (m, 4H); ESI-MS: m/z Calculated 470.48, found [M+H]$^+$ 471.4; Anal. Calcd for C$_{19}$H$_{16}$ClN$_{5}$O$_{4}$S$_{2}$: C, 48.50; H, 3.00; N, 17.86; O, 17.00; S, 13.63 %; found C, 48.70; H, 3.18; N, 17.65; S, 13.79 %.

N-(chlorobenzo[d]thiazol-2-yl)-2-(2-(6-chloroquinolin-4-yl)hydrazinyl)acetamide (5f): IR (KBr) ($\nu_{\text{max}}$ cm$^{-1}$): 3045 (C-H Aromatic), 3379 (N-H), 1450 (C=O amide); $^1$H NMR (400 MHz, DMSO) $\delta$ 12.01 (s, 1H), 9.79 (s, 1H), 8.16 (d, $J = 1.4$ Hz, 1H), 7.88 (d, 1H), 7.41 (dd, 1H), 7.36 – 7.25 (m, 2H), 7.23 – 7.08 (m, 2H), 4.07 (s, 1H), 3.56 – 3.43 (m, 3H), 3.03 (s, 1H); ESI-MS: m/z Calculated 459.93, found [M+H]$^+$ 460.9. Anal. Calcd for C$_{19}$H$_{14}$ClN$_{5}$O$_{4}$S$_{2}$: C, 49.62; H, 3.07; Cl, 7.71; N, 15.23; O, 10.44; S, 13.94 %; found C, 49.89; H, 3.25; N, 15.35; S, 13.65 %.

2-(2-(6-chloroquinolin-4-yl)hydrazinyl)-N-(6-ethoxybenzo[d]thiazol-2-yl)acetamide (5g): IR (KBr) ($\nu_{\text{max}}$ cm$^{-1}$): 3072 (C-H Aromatic), 3385 (N-H), 1486 (C=O amide), 1672 (C=O amide); $^1$H NMR (400 MHz, DMSO) $\delta$ 11.00 (s, 1H), 9.84 (s, 1H), 8.01 (d, $J = 7.5$ Hz, 1H), 7.91 (d, 1H), 7.37 – 7.23 (m, 3H), 7.21 – 6.20 (m, 2H), 4.94 (s, 1H), 4.03 (q, 2H), 3.59 – 3.45 (m, 4H), 1.39 (t, 3H); ESI-MS: m/z Calculated 469.54, found [M+H]$^+$ 470.5. Anal. Calcd for C$_{21}$H$_{19}$N$_{5}$O$_{4}$S$_{2}$: C, 53.72; H, 4.08; N, 14.92; O, 13.63; S, 13.66 %; found: C, 53.96; H, 4.15; N, 14.65; S, 13.45 %.

**Determination of antibacterial activity**

Antibacterial activities of 5a-5g were carried out in Nutrient-agar plates by well diffusion assay. Cultures were activated in Nutrient broth. Isolates were inoculated in Nutrient broth and
incubated at 37 °C for 24 hours for activation of cultures and then centrifuged at 3000 rpm for 15 min and the supernatant was collected to study antibacterial activity.

Using in-vitro agar well diffusion method, antimicrobial activity experiments were carried out. The activity of 5a-5g against test microorganisms (1000 μl volume with 1000 μg/ml concentration) of activated test cultures 2 Gram negative and 2 Gram positive; viz. Enterobacter aerogens MTCC No. 8558, Escherichia coli MTCC No. 1610, Micrococcus luteus MTCC No. 11948 and Bacillus cereus MTCC No. 8557) was inoculated in molten agar and poured into sterile plates than allowed to solidify. Wells with 5mm diameter were prepared at equal distance in solidified agar plates using cup-borer. Various derivatives 5a-5g with 1000 μgm/ml concentration were inoculated in the wells of nutrient agar whereas test microorganisms were inoculated by pour plate technique. The plates were incubated at 37 °C for 24 hours. The inhibition zones were measured at the end of the incubation period.

3. RESULT AND DISCUSSION

Chemistry

The synthetic pathway for preparation of different derivative of N-(benzo[2]thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'yl)amino)acetamide is shown in Scheme 1. To explore the scope of spirowxindole, we extended our studies to the use of various substituted 2-aminobenzothiazole and chloroacetyl chloride in the presence of triethyl amine and in sequence there is a linkage with hydrazine hydrate. This condensation proceeds probably by way of isatin in ethanol to generate Schiff’s base. Moreover, excellent results were obtained when the reaction was further proceed for cyclisation with thioglycolic acid to construct spiro[indoline-3,2'-thiazolidin] nucleus. It was gratifying to observe that most of the tested substrates exhibited satisfactory reactivity profiles, in microwave for 5-7 min at 350 W in all cases leading to readily afford the target structures (Table 1).
A series of compound 4a–4g has been synthesized by conventional method and a series of compound 5a–5g has been synthesized by microwave irradiation method as illustrated in scheme 1. The structures of all the newly synthesized compounds were confirmed by elemental analysis and with FT-IR, $^1$H NMR, and Mass analysis. The IR spectra of compound 5a shows four main peaks at 3078, 3289, 1560, 1655 $\nu_{\text{max}}$, cm$^{-1}$ of C-H Aromatic, N-H, C=C aromatic, C=O amide respectively. In $^1$H NMR spectrum of 5a shows characteristic peaks at 12.04 and 9.79 $\delta$ value resulting from -NH of amide group. Aromatic proton of targeted compound resonates at 8.19 – 7.08 $\delta$ ppm. The mass spectrum of selected compound 5a showed [M+H]$^+$ peak at m/z 426.5. The appearance of a molecular ion peak at 426.5 mass unit supports the structure of compound 5a.

**Antibacterial Activity**

All the synthesized compounds were screened for their antibacterial activity out on Nutrient-agar plates by well–diffusion assay against test culture. Cultures were activated in Nutrient broth. Isolates inhibits the above mentioned organisms or not were studied. Zone of inhibition was measured in terms of zone diameter and with the help of that zone index was calculated by using streptomycin as a standard drug. Compounds 6a–6i were screened against *Enterobacter aerogens* (MTCC 8558), *Escherichia coli* (MTCC 1610), *Micrococcus luteus* (MTCC 11948), and *Bacillus cereus* (MTCC 8558). The results are illustrated in Table 3.

**Determination of activity index**

The activity index of the probiotic culture was calculated as:

$$ \text{Activity index (A.I.)} = \frac{\text{Mean of zone of inhibition of derivative}}{\text{Zone of inhibition obtained for standard antibiotic drug}} $$

Note: Standard drug used was Streptomycin with 1000 µg/ml concentration.
The synthesized compounds 5a–5g were tested in vitro for antibacterial activity against Gram-negative Enterobacter aerogens (MTCC No. 8558), Escherichia coli (MTCC No. 1610), and Gram-positive Micrococcus luteus (MTCC No. 11948), Bacillus cereus (MTCC No. 8558) by measuring the zone of inhibition in mm. Antibacterial screening results (the zone of inhibition), presented in Table 2, revealed that all compounds tested showed some degree of antibacterial activity. The antibacterial activity data revealed that almost all the compounds 5a–5g exhibited favourable antibacterial activity against gram negative and gram positive bacteria compared to streptomycin. Compounds 5c and 5g exhibited better zone of inhibition against Enterobacter aerogens and Escherichia coli gram negative bacteria respectively. For Micrococcus luteus, compounds 5e found to be very active than all other compounds in the series. Moreover, compounds 5e indicated good activity against Bacillus cereus gram positive bacteria.

| Derivatives | Enterobacter aerogens MTCC No. 8558 | Escherichia coli MTCC No. 1610 | Micrococcus luteus MTCC No. 11948 | Bacillus cereus MTCC No. 8558 |
|------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|            | Mean value for Zone of Inhibition (mm) | Activity Index (A.I.) | Mean value for Zone of Inhibition (mm) | Activity Index (A.I.) | Mean value for Zone of Inhibition (mm) | Activity Index (A.I.) | Mean value for Zone of Inhibition (mm) | Activity Index (A.I.) |
| 5a         | 23 | 0.958 | 24 | 1.000 | 17 | 0.703 | 23 | 0.958 |
| 5b         | 19 | 0.791 | 22 | 0.916 | 22 | 0.916 | 25 | 1.041 |
| 5c         | 26 | 1.083 | 24 | 1.000 | 19 | 0.791 | 27 | 1.125 |
| 5d         | 22 | 0.916 | 21 | 0.875 | 24 | 1.000 | 19 | 0.791 |
| 5e         | 23 | 0.958 | 20 | 0.833 | 28 | 1.166 | 24 | 1.000 |
| 5f         | 21 | 0.875 | 21 | 0.875 | 19 | 0.791 | 21 | 0.875 |
| 5g         | 17 | 0.703 | 26 | 1.083 | 23 | 0.958 | 23 | 0.958 |
| Std drug   | 24 | --- | 24 | --- | 24 | --- | 24 | --- |

4. CONCLUSION

We have presented a series of N-(benzo[d]thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'yl)amino)acetamide derivatives that have been synthesized by sequencing reaction. It has been synthesized successfully in substantial yields and from all the screened compounds for in vitro antibacterial activity study, Compounds 5c, 5e and 5g appeared as the most active antibacterial agent against gram positive and negative bacteria as compared to streptomycin. Most importantly of all, that newly synthesized derivatives can be used for the development of new antibacterial drugs for the treatment of many disorders caused by the different bacterial species.
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References

[1] (a) T. Torroba, Poly-sulfur-nitrogen heterocycles via sulfur chlorides and nitrogen reagents, J. prakt. Chem., 341 (1999) 99-113; (b) V. Polshettiwar, RS. Varma, Microwave-assisted organic synthesis and transformations using benign reaction media, Acc. Chem. Res. 41 (2008) 629-639; (c) D. Garella, E. Borretto, A. Di Stilo, K. Martina, G. Cravotto, P. Cintas, Microwave-assisted synthesis of N-heterocycles in medicinal chemistry, MedChemComm, 4 (2013) 1323-1343.

[2] MN. Bhoi, MA. Borad, HD. Patel, Synthetic strategies for fused benzothiazoles: past, present and future, Synth. Commun., 44 (2014) 2427-2457.

[3] T. Jiang, KL. Kuhen, K. Wolff, H. Yin, K. Bieza, J. Caldwell, B. Bursulaya, T. Tuntland, K. Zhang, D. Karanewsky, Y. H, Design, synthesis, and biological evaluations of novel oxindoles as HIV-1 non-nucleoside reverse transcriptase inhibitors. Part 2, Bioorg. Med. Chem. Lett., 16 (2006) 2109–2112.

[4] M. Verma, S. Nath Pandeya, K. Nand Singh, JP. Stables, Anticonvulsant activity of Schiff bases of isatin derivatives, Acta Pharm., 54 (2004) 49–54.

[5] A. Amal Raj, R. Raghunathan, MR. Sridevikumaria, N. Raman, Synthesis, antimicrobial and antifungal activity of a new class of spiro pyrrolidines, Bioorg. Med. Chem., 11 (2003) 407–419.

[6] R. Tripathy, A. Reiboldt, PA. Messina, M. Iqbal, J. Singh, ER. Bacon, TS. Angeles, SX. Yang, MS. Albom, C. Robinson, H. Chang, BA. Ruggeri, JP. Mallamo, Structure-guided identification of novel VEGFR-2 kinase inhibitors via solution phase parallel synthesis, Bioorg. Med. Chem. Lett., 16 (2006) 2158–2162.

[7] T. Ratan Bal, B. Anand, P. Yogeewari, DH. Sriram, Synthesis and evaluation of anti-HIV activity of isatin β-thiosemicarbazone derivatives, Bioorg. Med. Chem. Lett. 15 (2005) 4451–4455.

[8] G. Cecile, D. Douguet, V. Huteau, M. Gilles, ML. Helene, P. Sylvie, Substituted benzylpyrimidines targeting thymidine monophosphate kinase of Mycobacterium tuberculosis: synthesis and in vitro anti-mycobacterial activity, Bioorg. Med. Chem. 16 (2008) 6075-6085.

[9] R. Lin, G. Sigmond, PJ. Johnson, SK. Connolly, E. Wetter, TV. Binnun, WV. Hughes, NB. Murray, SJ. Pandey, MM. Mazza, AR. Adams, F. Pesquera, AM. Steven, Synthesis and evaluation of 2, 7-diamino-thiazolo [4, 5-d] pyrimidine analogues as anti-tumor epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, Bioorg. Med. Chem. Lett., 19 (2009) 2333-2337.

[10] (a) Q. Chen, X. Zhu, L. Jiang, LM. Yang, G. Fu, Synthesis, antifungal activity and CoMFA analysis of novel 1, 2, 4-triazolo [1, 5-a] pyrimidine derivatives, Eur. J. Med. Chem., 43 (2008) 595-603. (b) MMM. Santos, Recent advances in the synthesis of biologically active spirooxindoles, Tetrahedron, 70 (2014) 9735-9757.

[11] MA. Borad, MN. Bhoi, NP. Prajapati, HD. Patel, A Review on Synthesis of Spiro Heterocyclic Compounds from Isatin, Synth. Commun., 44 (2014) 897-922.
[12] MA. Borad, MN. Bhoi, NP. Prajapati, HD. Patel, A Review on Synthesis of multi Spiro Heterocyclic Compounds from Isatin, Synth. Commun., 44, (2014) 1-15.

[13] MA. Borad, MN. Bhoi, DP. Rajani, SD. Rajani, HD. Patel, synthesis and antimicrobial activities of 2-(4-substituted phenyl)-1,10b dihydrospiro[benzo[e]pyrazolo[1,5-c][1,3]oxazine-5, 3'-indolin]-2'-one derivative from isatin, WJPPS, 3 (2014) 805-821.

[14] A. Agarwal, R. Ashutosh, N. Goyal, MS. Chauhana, S. Gupta, Dihydropyrido [2, 3-d] pyrimidines as a new class of antileishmanial agents, Bioorg. Med. Chem. 13 (2005) 6678-6684.