RESEARCH ARTICLE

Synthesis, Biological Evaluation and In-silico Studies of 3-((4-(6-(Substitutedphenyl)-2-Mercaptopyrimidin-4-Yl) Phenyl) Imino)-1-((Dimethylamino) Methyl) Indolin-2-One

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ABSTRACT

A novel isatin and pyrimidine prototype derivatives 3-((4-(6-(substitutedphenyl)-2-mercaptopyrimidin-4-yl)phenyl)imino)-1-((dimethylamino)methyl)indolin-2-one were synthesized and designed from the intermediate chalcones. The produced components were characterized, anti-tubercular and antimicrobial screening was done by agar dilution and agar-cup plate methods, respectively. From the studies, it was revealed that derivatives 5e, 5h and 5i showed increased potency. Further *in-silico* studies were carried out by molecular docking with the interaction of target components 5a-5j within the *Mycobacterium tuberculosis* enoyl-acp reductase enzyme (1ZID), drug likeness, and pharmacokinetic parameters were measured by osiris property explorer and molinspiration online toolkit respectively.

Keywords: Antitubercular activity, Chalcone, *Insilico* studies, Isatin, Pyrimidine.

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INTRODUCTION

Among the most prevalent infectious illnesses in the world is tuberculosis. After acquired immune deficiency syndrome (AIDS), it is the II major reason of mortality worldwide.[1] Despite the fact that tuberculosis has therapies, 1.5 million of the 9 million new cases recorded each year result in mortality. The third world has the worst epidemiological conditions, with incidence rates ranging from 100 to 300 cases per 100,000 persons.[2] Developing countries in Asia, Africa, Brazil, and Russia manage more than 95% of cases. Treatment outcomes are still inadequate. The success rate for new cases and operations described in 2013 was 76.0%.[3] Despite enormous exertions to treat and prevent tuberculosis, the problem remains serious. MDR-TB is a frightening phenomenon that includes isoniazid and rifampicin-resistant tuberculosis bacteria (multi-drug-resistant tuberculosis). Resistance to rifampicin, quinolones, aminoglycosides and isoniazid has also developed in mycobacteria, leading to extensively drug-resistant tuberculosis (XRD-TB).[4]

Due to the diverse inhabitants of mycobacteria (latent and active) and the ease with which resistance can be acquired, combination therapy combining antibiotics with multiple molecular mechanism of action is required.[5] As a result, treatment for multidrug-resistant tuberculosis is time-consuming, costly, and frequently futile. Another difficulty is an interaction of antivirals utilized in patient with Human immunodeficiency viruses (HIV) with anti-TB treatments.[6] As a result, tuberculosis challenges have captured the interest and efforts of many research organizations worldwide. To decrease the impact of *Mycobacterium tuberculosis* resistance, multifaceted attempts were made. A search for novel, potent anti-tubercular drug components are under underway. Because it is a time-consuming and costly procedure, it is vital to understand how effective antituberculosis drugs function. One approach to finding new potential drugs appears to be the development of equivalents with greater pharmacokinetic constraints and significantly less toxicity.

Isatin (1H-indole-2, 3-dione) is a chemical available in numerous organisms which is an endogenous nature. It is an adaptable structure for organic modification, its scaffolds exhibited assorted biological possessions such as antibacterial,[7,8] anticancer,[9] anti-HIV,[10] antimalarial,[11] anti-inflammatory,[12] antioxidant,[13] anticonvulsant,[14] anthelmintic,[15] and anti-TB actions.[16] Additionally, several isatin-based scaffolds include

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nintedanib, orantinib, sunitinib, and semaxanib have obtained clinical approval or are progressing through clinical studies. With a wide range of biological activity paired with a variety of structural changes and effective usage in clinical settings, more scientists investigate isatins and make many of their structurally diverse products derivatives.

Pyrimidine is a heterocyclic scaffold of interest to synthetic chemists because of its anti-cancer,[17] anti-fungal,[18] anti-protozoal,[19] anti-inflammatory,[20] anti-oxidant,[21,22] anti-estrogenic,[23] anti-platelet aggregation,[24] anti-malarial,[25] cardio-vascular actions,[26] and ease of synthesis. It was generally known that number of heterocyclic components, particularly those containing nitrogen, have a wide range of pharmacological actions. In light of pyrimidine's results regarding its activities, and as part of our ongoing attempts to develop anti-tubercular medicines, we designed and synthesized some innovative prototypes that take the benefit of 2 pharmacophores of pyrimidine and isatin in the backbone of single molecule.

MATERIALS AND METHODS

General

All chemical constituents were of spectroscopic grade and utilized as such, lacking further purification in the synthesizing, which were attained from commercial standard sources. Utilizing silica gel G as an adsorbent from Merck and solvent mixtures mentioned where necessary, reactions were observed utilizing TLC. Silica gel (100 to 200 mesh, Merck grade) has been utilised for column chromatography. 100 *mL* of each portion was collected. Under a UV light, the TLC isolations of the components were examined. The most prevalent infectious illnesses in the world is tuberculosis. After AIDS, it is the II major reason of mortality worldwide

Using the melting point instrument (Boitus digital), the melting point of all were calculated in open capillary tubes, given in ºC, and were not corrected. Tetramethylsilane [$(CH_3)_4Si$] was used as and internal standard to record ^{1}H-NMR spectrum of the components in CDCl3 to use a Bruker Ultra Shield NMR (400 MHz) spectrometer. In ppm, chemical shift (δ) is measured.

Employing electron impact ionisation and a JEOL-SX-102 equipment, the mass spectrums were acquired. On a Jasco 410-fourium transform infrared spectrometer, all of the IR spectrums were captured in pellets of KBr. The results of elemental studies using a model of Perkin Elmer-240c analyzer were less than 0.40% of predicted findings.

General method for Synthesising (Z)-3-((4-((E)-3-(substitutedphenyl)-3-oxoprop-1-en-1-yl) phenyl)imino) indolin-2-one (3a-3j)

In existence of glacial acetic acid, equimolar amounts of isatin (0.01 mol) and p-amino benzaldehyde were dissolved in enough methanol (30 *mL*), refluxed for 1hr, and then left at 37ºC (room) for 2 hours. The separated precipitate was filtered, vacuum-dried, and reconstituted from pure ethanol.

Equimolar quantities (0.01 mol) of the obtained precipitate ((Z)-4-((2-oxoindolin-3-ylidene)amino)benzaldehyde) and various aromatic ketones were allowed for stirring with the help of magnetic stirrer using ethanol as solvent at ambient temperature. Sodium hydroxide was used as a catalyst. From a (1:1) DMF/EtOH combination, the solid was extracted by filtering, cleaned, and then recrystallized (Scheme 1).

(Z)-3-((4-((E)-3-(3-chlorophenyl)-3-oxoprop-1-en- 1-yl) phenyl)imino)indolin-2-one (3a)

Yield = 72 %, MP 158-160°C. **^{1}H-NMR (400 MHz, $CDCl_3$) δppm:** 8.06-8.14 (d, 1H, HC=CH-CO), 7.60-7.69(d, 1H, CH=CH-CO), 7.24-7.96(m, 12H, Ar-CH), 10.31(s, 1H, NH). **IR(KBr)cm^{-1}:** 3025 (HC=CH), 2946(Ar-CH), 1674 (C=N), 1741 (O=C), 1213 (C-Cl), 1587 (C=C). **EI-MS *m/z*:** 386 (M^+). *Anal*. Calcd for $C_{25}H_{18}F_2N_2O$: C, 71.41; H, 3.91; N, 7.24; Found:C, 71.31; H, 3.83; N,7.16

(Z)-3-((4-((E)-3-(3-nitrophenyl)-3-oxoprop-1-en-1-yl)phenyl) imino)indolin-2-one (3b)

Yield=73%, MP. 182-184 °C. **^{1}H-NMR(CCl_3D, 400MHz) δppm:** 8.07-8.12(d, 1H, HC=CH-CO), 7.03-7.12(d, 1H, HC=CH-CO), 7.17-8.54(m, 12H, Ar-CH), 10.26 (s, 1H, NH). **IR(KBr)cm^{-1}:** 3024 (HC=CH), 2946 (Ar-CH), 1740 (O=C), 1647 (N=C), 1601 (C=C), 1540 & 1370 (NO_2). **EI-MS *m/z*:** 397(M^+). *Anal*. Calcd for $C_{23}H_{15}N_3O_4$: C,69.52; H, 3.80; N,10.57; Found C,69.29; H,3.72; N,10.51.

(Z)-3-((4-((E)-3-(3-methoxyphenyl)-3-oxoprop-1-en-1-yl) phenyl)imino)indolin-2-one (3c)

Yield=81%, MP 150 to 152°C. **^{1}H-NMR($CDCl_3$, 400MHz) δppm:**8.86-8.96(d, 1H, HC=CH-CO), 8.40-8.52(d, 1H, HC=CH-CO), 7.05-8.10(m, 12H, Ar-CH), 3.63(s, 3H, OCH_3), 10.35(s, 1H, NH). **IR(KBr)cm^{-1}:** 3014(HC=CH), 2950(Ar-CH), 1734 (O=C), 1670 (N=C), 1614(C=C) & 1013 (C-O-C). **EI-MS*m/z*:** 382 (M^+). *Anal*. Calcd for $C_{24}H_{18}N_2O_3$: C, 75.38; N, 7.33; H, 4.74; Found: C, 75.31; H,4.67; N,7.23.

(Z)-3-((4-((E)-3-(4-aminophenyl)-3-oxoprop-1-en-1-yl) phenyl)imino)indolin-2-one (3d)

Yield=80%, MP 161-163°C. **^{1}H-NMR ($CDCl_3$, 400 MHz) δppm:** 9.03-9.12(d, 1H, HC=CH-CO), 8.47-8.60 (d, 1H, HC=CH-CO), 10.40 (s, 1H, NH), 5.14 (s, 2H, NH_2), 7.15-8.12 (m, H12, Ar-CH), 5.14 (s, 2H, NH_2), 10.40 (s, 1H, NH). **IR(KBr)cm^{-1}:**3017 (HC=CH), 2950(Ar-CH), 1735 (O=C), 1661(N=C), 1626(C=C). **EI-MS *m/z*:**367(M^+). *Anal*. Calcd for $C_{23}H_{17}N_3O_2$: H, 4.66; N, 11.44; C, 75.19; Found: C, 75.02; H, 4.55; N, 11.40.

(Z)-3-((4-((E)-3-(4-dimethylaminophenyl)-3-oxoprop-1-en-1-yl)phenyl)imino) indolin- 2-one (3e)

Yield=79%, MP 172-174 °C. **^{1}H-NMR ($CDCl_3$, 400 MHz) δppm:** 9.13-9.30(d, 1H, HC=CH-CO), 8.59-8.70(d, 1H, HC=CH-CO), 6.83-7.88(m, 12H, Ar-CH), 3.12(s, 6H, N-$(CH_3)_2$), 10.32(s, 1H, NH). **IR(KBr)cm^{-1}:**3075(CH=CH), 2919(Ar-CH), 1735 (O=C), 1645 (N=C), 1608 (C=C). **EI-MS *m/z*:**395.46 (M^+). *Anal*. Calcd for $C_{25}H_{21}N_3O_2$: C, 75.93; H, 5.35; N, 10.63; Found: 75.90; H, 5.28; N, 10.55.

(Z)-3-((4-((E)-3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl) phenyl)imino)indolin-2-one (3f)

Yield=84%, MP 185-187 °C. **^{1}H-NMR ($CDCl_3$, 400 MHz) δppm:** 8.48-8.59(d, 1H, HC=CH-CO), 7.76-7.89(d, 1H, HC=CH-CO), 6.68-7.71(m, 12H, Ar-CH), 10.36 (s, 1H, NH). **IR(KBr)cm^{-1}:** 3085(CH=CH), 3022(Ar-CH), 1708 (O=C), 1659 (N=C), 1613(C=C), 1213(C-F) and 819(C-Cl). **EI-MS *m/z*:** 386 (M^+). *Anal*. Calcd for $C_{23}H_{15}ClN_2O_2$: C, 71.41; H, 3.91; N, 7.24; Found: C, 71.37; H, 3.90; N, 7.12.

(Z)-3-((4-((E)-3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl) phenyl)imino) indolin-2-one (3g)

Yield=69%, MP 148 to 150 °C. **^{1}H-NMR($CDCl_3$, 400 MHz) δppm:** 9.18-9.28(d, 1H, HC=CH-CO), 8.45-8.56(d, 1H, HC=CH-CO), 7.12-8.09(m, 11H, Ar-CH), 3.89(s, 3H, OCH_3), 3.38 (s, 3H, OCH_3), 10.29 (s, 1H, NH). **IR(KBr) cm^{-1}:**3075(HC=CH), 3026(Ar-CH), 1728 (O=C), 1654 (N=C), 1605 (C=C), 1028 (C-O-C). **EI-MS *m/z*:** 413(M^{+1}). *Anal*. Calcd for $C_{25}H_{20}N_2O_4$: C,72.80; H,.89; N,6.79; Found: C,.74; H,4.81; N,6.72.

(Z)-3-((4-((E)-3-(2-bromophenyl)-3-oxoprop-1-en-1-yl) phenyl)imino)indolin-2-one (3h)

Yield = 75 %, MP 182 to 184°C. **^{1}H-NMR ($CDCl_3$, 400 MHz) δppm:** 8.59-8.68(d, 1H, HC=CH-CO), 7.74 to 7.87(d, 1H, HC=CH-CO), 6.92 to 7.86(m, 12H, Ar-CH), 10.25(s, 1H, NH). **IR(KBr)cm^{-1}:**3075(HC=CH), 3024(Ar-CH), 1735(O=C), 1644(N=C), 1608(C=C), 958(C-Br). **EI-MS *m/z*:** 396(M). *Anal*. Calcd for $C_{23}H_{15}BrN_2O_2$: C,64.05; H,3.51; N,6.50;; Found: C,63.93; H,3.42; N,6.46.

(Z)-3-((4-((E)-3-(4-bromophenyl)-3-oxoprop-1-en-1-yl) phenyl)imino)indolin-2-one (3i)

Yield=77%, MP 170 to 172 °C. **^{1}H-NMR(CCl_3D, 400MHz) δppm:** 8.80(d, 1H, HC=CH-CO), 7.60(d, 1H, HC=CH-CO), 7.23-8.11(m, 12H, Ar-CH), 10.34(s, 1H, NH). **IR(KBr) cm^{-1}:**3080(HC=CH), 3024(Ar-CH), 1717(O=C), 1655(N=C), 1620(C=C), 962(C-Br). **EI-MS *m/z*:**431 (M^+). *Anal*. Calcd for $C_{23}H_{15}BrN_2O_2$: C, 64.05; H, 3.51; N, 6.50: Found: C, 63.97; H, 3.42; N, 6.45

(Z)-3-((4-((E)-3-(4-florophenyl)-3-oxoprop-1-en-1-yl)phenyl) imino)indolin-2-one (3j)

Yield=78%, MP 180-182 °C. **^{1}H-NMR (CCl_3D, 400MHz) δppm:** 8.78(d, 1H, HC=CH-CO), 7.94(d, 1H, HC=CH-CO), 7.14-7.91(m, 12H, Ar-CH), 10.33(s, 1H, NH). **IR(KBr)cm^{-1}:** 3081(HC=CH), 3017(Ar-CH), 1735(O=C), 1640(N=C), 1622(C=C), 1225(C-F). **EI-MS *m/z*:** 370(M^+), *Anal*. Calcd for $C_{23}H_{15}FN_2O_2$: C,74.59; H, 4.08; N,7.56; Found: C,74.52; H,3.98; N,7.48. **IR(KBr)cm^{-1}:** 3081(HC=CH), 3017(Ar-CH), 1735(O=C), 1640(N=C), 1622(C=C), 1225(C-F).

Preparation of (Z)-3-((4-(6-(substitutedphenyl)-2-mercaptopyrimidin-4-yl)phenyl) imino)-1-((dimethylamino)methyl)indolin-2-one (5a-5j)

The above synthesized (Z)-3-((4-((E)-3-(substituted phenyl)-3-oxoprop-1-en-1-yl)phenyl)imino)indolin-2-one (1 mmol) derivatives were refluxed with 1-chloro-*N,N*-dimethyl methanamine and thiourea upto 8 to 10 hours in presence of 20 *mL* of acetic acid. Additionally, TLC was utilised to keep track of the response, and vacuum evaporated the solvent. After the reaction was finished, the residue was well mixed before the crushed ice was added. Under vacuum, the precipitate was filtered. The analytical pure compound shown in Scheme 1 was produced by recrystallizing the crude compounds from an appropriate solvent.

(Z)-3-((4-(6-(3-chlorophenyl)-2-mercaptopyrimidin-4-yl) phenyl)imino)-1-((dimethyl amino)methyl)indolin-2-one (5a)

Yield=68%, MP. 210-214°C. **EI-MS *m/z*:** 499(M^+), *Anal*. Calcd for $C_{27}H_{22}ClN_5OS$: C,64.86; H,4.44; N,14.01; Found: C,64.81; H,4.34; N,13.93. **^{1}H-NMR($CDCl_3$, 400 MHz)δppm:** 12.05(s, 1H, SH), 2.38(s, 6H, N-$(CH_3)_2$), 4.70(s, 2H, N-CH_2-N), 8.6(s, 1H, CH-Pyrimidine), 7.28-8.1(m, 12H, Ar-H).

(Z)-3-((4-(6-(3-nitrophenyl)-2-mercaptopyrimidin-4-yl) phenyl)imino)-1-((dimethyl amino)methyl)indolin-2-one (5b)

Yield=66%, MP 232-236°C. **EI-MS *m/z*:** 510(M^+), *Anal*. Calcd for $C_{27}H_{22}ClN_5OS$: C,63.52; H,4.38; N,16.46; Found: C, 63.47; H, 4.32; N, 16.38. **^{1}H-NMR($CDCl_3$, 400 MHz)δppm:** 12.15(s, 1H, SH), 2.32(s, 6H, N-$(CH_3)_2$), 4.66(s, 2H, N-CH_2-N), 8.60(s, 1H, CH-Pyrimidine), 7.18-8.1(m, 12H, Ar-H).

(Z)-3-((4-(6-(3-methoxyphenyl)-2-mercaptopyrimidin-4-yl) phenyl)imino)1-((dimethyl amino)methyl)indolin-2-one (5c)

Yield=60%, MP 226-230°C. **EI-MS *m/z*:**495(M^+), *Anal*. Calcd for $C_{28}H_{25}N_5O_2S$: C,67.86; H,5.08; N,14.13; Found: C, 67.69; H, 5.01; N, 14.05. **^{1}H-NMR(CCl_3D, 400MHz)δppm:** 12.15(s, 1H, SH), 2.35(s, 6H, N-$(CH_3)_2$), 4.72(s, 2H, N-CH_2-N), 3.62(s, 3H, O-CH_3), 8.41(s, 1H, CH-Pyrimidine), 7.12 to 8.02(m, 12H, Ar-H).

(Z)-3-((4-(6-(4-aminophenyl)-2-mercaptopyrimidin-4-yl) phenyl)imino)-1-((dimethyl amino)methyl)indolin-2-one (5d)

Yield=63%, MP 241-245°C. **EI-MS *m/z*:** 480 (M^+), *Anal*. Calcd for $C_{27}H_{24}N_6OS$: C, 67.48; H, 5.03; N, 17.49; Found: C, 67.41; H, 5.01; N, 14.0. **^{1}H-NMR(CCl_3D, 400MHz)δppm:**12.25(s, 1H, SH), 2.26(s, 6H, N-$(CH_3)_2$), 4.68(s, 2H, N-CH_2-N), 5.31(s, 3H, C-NH_2), 8.31(s, 1H, CH-Pyrimidine), 7.28-8.0 (m, 12H, Ar-H).

(Z)-3-((4-(6-(4-dimethylaminophenyl)-2-mercaptopyrimidin-4-yl) phenyl)imino)1-((dimethyl amino) methyl)indolin-2-one (5e)

Yield=68%, MP 248-252°C. **EI-MS *m/z*:** 508 (M^+), *Anal*. Calcd for $C_{29}H_{28}N_6OS$: C, 68.48; H, 5.55; N, 16.52; Found: C, 68.41; H, 5.51; N, 16.40. **^{1}H-NMR($CDCl_3$, 400 MHz) δppm:** 12.08(s, 1H, SH), 3.12(s, 6H, C-N-$(CH_3)_2$), 2.22(s, 6H, N-$(CH_3)_2$. 4.59(s, 2H, N-CH_2-N), 5.31(s, 3H, C-NH_2), 8.38(s, 1H, CH-Pyrimidine), 7.31-8.06(m, 12H, Ar-H).

(Z)-3-((4-(6-(4-chlorophenyl)-2-mercaptopyrimidin-4-yl) phenyl)imino)-1-((dimethyl amino)methyl)indolin-2-one (5f)

Yield=70%, MP 222-226°C. **EI-MS *m/z*:** 499(M^+), *Anal*. Calcd for $C_{27}H_{22}ClN_5OS$: C, 64.86; H, 4.44; N, 14.01; Found: C, 64.81; H, 4.42; N, 16.35. **^{1}H-NMR($CDCl_3$, 400 MHz)δppm:** 12.10(s, 1H, SH), 2.26(s, 6H, N-$(CH_3)_2$. 4.61(s, 2H, N-CH_2-N), 8.42(s, 1H, CH-Pyrimidine), 7.12-8.12 (m, 12H, Ar-H).

(Z)-3-((4-(6-(3,4-dimethoxyphenyl)-2-mercaptopyrimidin-4-yl)phenyl)imino)-1-((dimethylamino)methyl)indolin-2-one (5g)

Yield=65%, MP 242-246°C. **EI-MS *m/z*:** 525 (M^+), *Anal*. Calcd for $C_{27}H_{28}N_5O_3S$: C, 66.27; H, 5.18; N, 13.32; Found: C, 66.17; H, 5.12; N, 13.25. **^{1}H-NMR($CDCl_3$, 400 MHz)δppm:** 12.15(s, 1H, SH), 2.18(s, 6H, N-$(CH_3)_2$. 3.32(s, 6H, O-CH_3), 4.52(s, 2H, N-CH_2-N), 8.36(s, 1H, CH-Pyrimidine), 7.02-8.02(m, 12H, Ar-H).

(Z)-3-((4-(6-(2-bromophenyl)-2-mercaptopyrimidin-4-yl) phenyl)imino)-1-((dimethyl amino)methyl)indolin-2-one (5h)

Yield=70%, MP 233-237°C. **EI-MS *m/z*:** 543 (M^+), *Anal*. Calcd for $C_{27}H_{22}BrN_5OS$: C, 59.56; H, 4.07; N, 12.86; Found: C, 59.53; H, 4.06; N, 12.83. **^{1}H-NMR($CDCl_3$, 400 MHz)δppm:** 12.02(s, 1H, SH), 2.18(s, 6H, N-$(CH_3)_2$, 4.68(s, 2H, N-CH_2-N), 8.51(s, 1H, CH-Pyrimidine), 7.12-7.93(m, 12H, Ar-H).

(Z)-3-((4-(6-(4-bromophenyl)-2-mercaptopyrimidin-4-yl) phenyl)imino)-1-((dimethyl amino)methyl)indolin-2-one (5i)

Yield=73%, MP 233-237°C. **EI-MS *m/z*:** 543 (M^+), *Anal*. Calcd for $C_{27}H_{22Br}N_5OS$: C, 59.56; H, 4.07; N, 12.86; Found: C, 59.53; H, 4.06; N, 12.83. **^{1}H-NMR($CDCl_3$, 400 MHz)δppm:** 12.04(s, 1H, SH), 2.18(s, 6H, N-$(CH_3)_2$, 4.69(s, 2H, N-CH_2-N), 8.48 (s, 1H, CH-Pyrimidine), 7.12-7.98 (m, 12H, Ar-H).

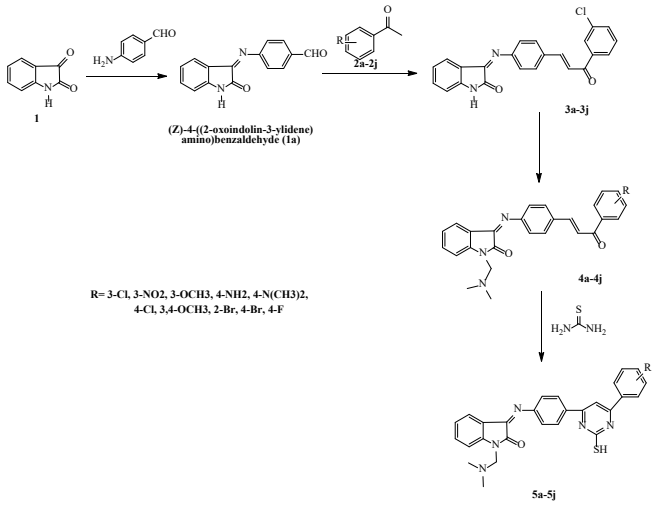
(Z)-3-((4-(6-(4-florophenyl)-2-mercaptopyrimidin-4-yl) phenyl)imino)-1-((dimethyl amino)methyl)indolin-2-one (5j)

Yield=72%, MP 241-245°C. **EI-MS *m/z*:** 483 (M^+), *Anal*. Calcd for $C_{27}H_{22}FN_5OS$: C, 67.06; H, 4.59; N, 14.48; Found: C, 67.01; H, 4.52; N, 14.42. **^{1}H-NMR($CDCl_3$, 400 MHz)δppm:** 12.04(s, 1H, SH), 2.26(s, 6H, N-$(CH_3)_2$, 4.72(s, 2H, N-CH_2-N), 8.46(s, 1H, CH-Pyrimidine), 7.14-8.01(m, 12H, Ar-H) (Scheme 1).

Pharmacological Screening

In-vitro Anti-tubercular action

Title compounds' anti-tubercular efficacy was calculated using the agar dilution technique (an *in-vitro M. tuberculosis* approach) [1-2]. Each test analog was introduced onto a Middle Brook/7H11 agar slant using OADC supplement growth in ten fold serial dilution. Using new Middle Brook/7H11 agar slant made to 1 mg/*mL* in 0.05% tween 80 saline dilute to 10^{-2} (about 10^7 cfu/mL concentrations), *M. tuberculosis* H37RV inoculums were created. Ten fold serial dilution of test analogue samples were added to 7H11 agar tubes per mL along with 5 µL of bacterial suspension. The tubes were incubated at 37°C, and 28 days later, the final findings were taken. Test tube findings (Test analogue, medium, and $H_{37}RV$) were equated to control tube findings (Medium and $H_{37}RV$). MIC (minimum inhibitory concentration) is the concentration level at an *M. tuberculosis* growth completely inhibited. To compare the MIC of the title analogues, INH(isoniazid), pyrazinamide, rifampicin, and ethambutol were used as the reference drugs.



Scheme 1: Isatin synthesis and pyrimidine prototype derivative compounds.

In-vitro Antimicrobial Activity

The antibacterial action of test components (5a-5j) was examined by agar-cup plate technique (Miller and Rose, 1939). Dimethylsulfoxide was utilized as control and solvent. The solution of test and standard components were processed to get 1000 µg/mL concentration. The antimicrobial action of isatin and pyrimidine prototype derivatives were analysed and equated with ampicillin (standard) solutions at 3 dissimilar concentration levels i.e. 50.0, 100.0 and 150.0 µg/mL. Microbial strains of *Bacillus cereus*-ATCC 11778 and *Micrococcus luteus*- ATCC 4698 (Gram +ve bacteria), *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 11298 (Gram -ve bacteria), and fungal strains (*Aspergillus fumigatus* ATCC 46645) were taken for the studies

In-silico Screening

Molecular Docking Studies

All of the synthetic analogues (5a–5j) were stiff receptor docked, and the visual analysis of the resultant postures. The protein data bank was accessed, and the X-ray crystal structures of long fatty acid chains enoyl-acp reductase(INHA) in association with an isonicotinic-acyl-nadh blocker, PDB:1ZID, with resolution 2.70Å, was retrieved. Autodoc 4.2 software was used to conduct docking investigations. The Autodoc 4.2 programme was used to prepare the enzyme(Addition of polar H, Addition of AD4 type atoms, Remove of H_2O molecule and heteroatom). Using the previously acquired information of the actual ligand's interaction spot, binding site was identified. Each ligand was maintained flexible for the stimulation runs, but the active site aminoacid residues were kept stiff, and default parameter values were used. All of the compounds (5a–5j) inside the active site were computationally docked, and the findings show the energy related with inter-molecular contacts and hydrogen bonding interaction between functional groups of components and residues of amino acid.

Drug-likeness and Toxicity Prediction

The pharmaceutical integrity of the potential drug candidates was examined using Molinspiration online property osiris property explorer calculation toolbox. The Molinspiration online property calculation toolbox was utilized to estimate

Table 1: MIC *(µg/mL)* of synthesized isatin and pyrimidine prototype derivatives.

Compounds	*M. tuberculosis*
5a	3.90
5b	125.0
5c	62.50
5d	62.50
5e	1.95
5f	3.90
5g	31.25
5h	1.95
5i	1.95
5j	7.81
Isoniazid	0.12
Pyrazinamide	6.25
Rifampicin	0.12
Ethambutol	1.95

molecular descriptors number of H-bond acceptors, number of H-bond donors, the molecular weight of components, number of rotatable bonds and TPSA(topological polar surface area).

RESULTS AND DISCUSSION

Pharmacological Screening

In-vitro Anti-tubercular action

All title analogues were assessed for *in-vitro* antitubercular efficacy contrary to *M. tuberculosis* (strain H37Rv), and MIC of all of the tested analogues was calculated and shown in Table 1. To regulate the test organisms sensitivity, the MIC of rifampicin, ethambutol, and isoniazid were also assessed simultaneously. According to the findings, synthetic substances to varied degrees suppressed the development of *M. tuberculosis*. Analogs like 5e, 5h, and 5j, among other examined substances, suppressed the development of *M. tuberculosis* at a lower dose (1.95 g/mL), comparable to the common medication ethambutol. Components 5h and 5i have strong electron-withdrawing bromine atoms in the para and ortho positions on phenyl rings bonded to thiopyrimidine rings, respectively. Compound 5e has a dimethylamino group. The presence of a chlorine substituent in phenyl ring may be the reason why the MIC of test components 5a and 5f was determined to be 3.9 µg/mL. Even yet, the concentration at which derivative 5j with a fluorine moiety entirely prevented *M. tuberculosis* from growing was just 7.81 µg/mL. Test substances 5c and 5d's MICs were determined to be 62.50 µg/mL and 31.25 µg/mL, respectively, for analogues 5g. Compound 5b only showed action at higher concentrations (MIC: 125 µg/mL). It was discovered that the MIC for the common medications rifamipicin, ethambutol and isoniazid were 0.12, 1.95, and 0.12 µg/mL correspondingly.

Antimicrobial activity

Cup plate process was utilized to determine the *in-vitro* antimicrobial action of isatin and pyrimidine prototype derivatives 5a-5j which were depicted in Table 2. The activity of conventional medications (Fluconazole and Ampicillin) was measured in parallel tests and was compared with test compounds in order to regulate test organisms sensitivity. All of the investigated drugs shown moderate to excellent microbial suppression, according to the screening results.

According to the findings, B was superior to. In contrast to the remainder of the series, compound 5b, 5f, 5i, and compound 5j of the cereus showed reduced activity. While the other compounds all had inferior activity against M, compounds 5a, 5b, 5h, and 5i displayed activity that was equivalent to ampicillin. luteus. Every one of the compounds demonstrated the same strong anti-K action as the antibiotic ampicillin. except for 5c and 5g, pneumonia. The compounds tested against E had less action than expected. coli. The chemical 5i showed greater efficacy to the other tested derivatives against all bacteria among the other tested compounds.

The synthetic compounds' antifungal efficacies were tested on *A. fumigatus*. The chemicals mentioned in the title display varied levels of antifungal activity. Except for 5b and 5f, none of the synthesised compounds showed comparable or higher antifungal activity when tested against the tested fungi compared to conventional fluconazole. While the remainder of the title compounds exhibited low antifungal activity, compounds 5d, 5e, 5i, and 5j had demonstrated moderate antifungal activity.

Molecular Docking Studies

Using Autodock 4.2 software, docking experiments of the title compounds were conducted using enoyl-acp reductase in combination with inhibitory nature of isonicotinic-acyl-nadh, PDB: 1ZID. Figures 1 to 4 depict how a molecule interacts with the enzyme enoyl-acp reductase. The target compounds' docking data showed that all of them have positive energy properties in terms of Autodock score (Table 3). The most powerful compounds, 5e, 5h, 5i, and 5j, had Autodock scores of 10.5, 10.6, 11.0, and -10.09, respectively. When compared to the active compounds, the less active compounds' dock scores were shown to be higher. According to these findings, the less energetic compounds need more energy than the more energetic molecules for a successful

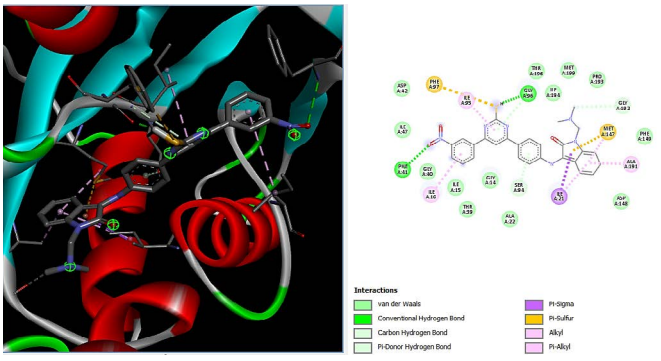


Figure 1: Docking poses of synthesized isatin and pyrimidine prototype derivative 5b with 1ZID protein.

Table 2: Antimicrobial activity of isatin and pyrimidine prototype derivatives (5a-5j).

Compound	Dose (µg/mL)	Antibacterial activity				
		Gram +ve bacteria		Gram-ive bacteria		Antifungal activity
		M. luteus	*B. cereus*	*E. coli*	*K. pneumoniae*	*A. fumigatus*
	50	-	-	-	-	-
5a	100	15	-	12	12	10
	150	22	12	16	22	15
	50	10	9	-	10	14
5b	100	16	18	-	17	20
	150	23	20	-	22	25
	50	-	-	-	-	10
5c	100	-	9	-	-	16
	150	-	16	-	-	20
	50	-	-	-	10	9
5d	100	-	9	10	15	14
	150	-	14	16	21	19
	50	-	-	12	11	-
5e	100	10	-	14	15	11
	150	19	17	16	23	15
	50	-	11	-	11	12
5f	100	11	17	-	17	17
	150	19	25	14	25	24
	50	-	-	-	-	-
5g	100	-	-	9	-	-
	150	-	-	15	-	-
	50	14	10	-	13	-
5h	100	16	14	12	17	15
	150	25	21	18	21	21
	50	11	12	12	-	11
5i	100	16	17	18	12	16
	150	23	24	26	20	23
	50	-	11	-	11	12
5j	100	9	16	14	16	17
	150	17	21	20	19	22
	50	14	14	14	15	14
Standard*	100	17	19	21	19	21
	150	26	27	27	25	27
Control	-	-	-	-	-	-

Table 3: Molecular Docking Study of synthesized isatin and pyrimidine prototype derivatives with 1ZID protein.

S.No.	Component	Docking energy (kcal/mol)	Number of H-bonds	Aminoacid interaction
1	5a	-9.9	2	Phe41 Gly96
2	5b	-9.6	2	Phe41 Gly96
3	5c	-9.7	4	Asp148 Ser94 Gly96 Val189
4	5d	-9.83	3	Ser94 Gly96 Val189
5	5e	-10.5	4	Asp148 Ser94 Gly96 Val189
6	5f	-9.9	2	Gly96 Val189
7	5g	-10.0	1	Val189
8	5h	-10.06	2	Phe41 Ile15
9	5i	-11.0	2	Phe41 Ile15
10	5j	-10.09	2	Phe41 Ile15

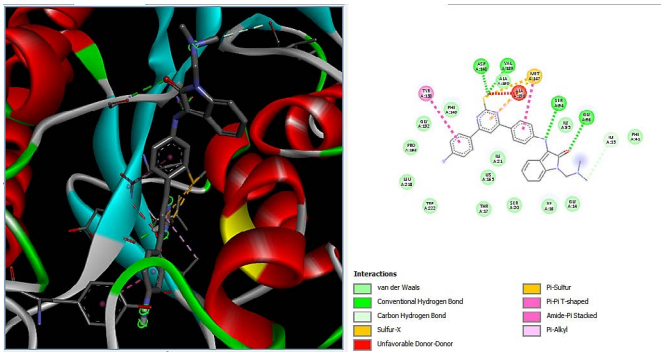


Figure 2: Docking poses of synthesized isatin and pyrimidine prototype derivative 5c with 1ZID protein.

binding contact with the receptor enzyme. The thiol substituted pyrimidine ring and 'N' atom of ring engage with the amino acid residues Phe41 and Ile15 by hydrogen bonding, according to the binding interaction of the most active molecule, 5i. Another active compound 5j shown interaction with amino acid Phe41, Ile15 through hydrogen bonding where as 5h showed similar hydrogen bond interaction with Phe41, Ile15 amino acid residues. All the interactions of isatin and pyrimidine prototype derivatives were depicted in Table 3**.** These binding interaction disclose importance of thiol group on the pyridine nucleus for favourable binding interaction with the receptors, so that better anti-tubercular activity is expected.

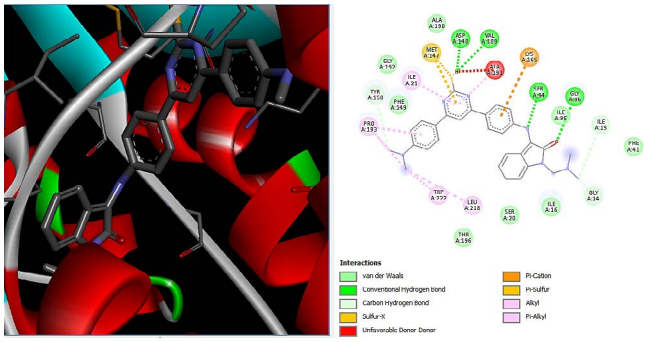


Figure 3: Docking poses of synthesized isatin and pyrimidine prototype derivative 5e with 1ZID protein.

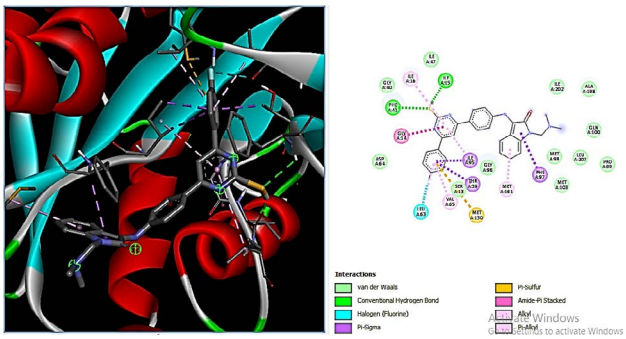


Figure 4: Docking poses of synthesized isatin and pyrimidine prototype derivative 5J with 1ZID protein.

Drug-likeness and toxicity prediction

Today, it is considerably easier to forecast the potential toxicity of a chemical using trustworthy bioinformatics methods. Utilizing the osiris property explorer, the current work computed the toxicity risks factors, including tumorigenicity, mutagenicity, irritability, and developmental or reproductive toxicity, for all the produced chemicals (5a–5j) (Table 4). Based on similarity of functional groups between the query derivative compounds and the *in-vivo* and *in-vitro* verified components in database of this online tool, predictions were made for the query derivatives. The toxicity risk predictor finds molecular fragments that could be hazardous by looking for possible toxicity risks. Color codes may be used to depict the findings; red designates a high propensity of toxicity, yellow shows a medium tendency of toxicity, and green shows a low tendency of toxicity. Compound 5e, however, showed a substantial risk of tumerogenic characteristics. In addition, TPSA analysis was used to assess the bioavailability of produced drugs.

This description has been connected to medication bioavailability and has been demonstrated to associate with passive transport of molecules via membrane, allowing expectation of drug transport features. According to Veber's rule, a drug's oral bioavailability is best when the number of rotatable bonds is at least ≤10, and the TPSA value is at least ≤140Å^2. (Veber *et al.*, 2002). It has been shown that number of rotatable bonding is an excellent indicator of the medications' oral bioavailability. Any one non-ring bond involved to a non-terminal heavy (i.e., non-H) atom is stated to as a rotatable bond. All of the components were determined to have an acceptable number of rotatable bonds. As per Zhao *et al.*, the equation %ABS=109-0.345 × TPSA was utilized to estimate the absorption percentage (Zhao *et al.*, 2002). Utilizing the Molinspiration online property determination toolbox and fragment-based technique proposed by Ertl *et al.*, TPSA was also determined (Ertl *et al.*, 2000).

Structural Activity Relationship

Title analogues 5e, 5h, and 5j showed excellent anti-tubercular activity overall in this research, whereas title analogues 5a, 5f, and 5j shown moderate anti-tubercular action. Other all title analogues just generated less anti-tubercular activity. The strong antibacterial action shown by derivatives 5e, 5h, and 5j may be due to the presence of halogen substituents like bromine, chlorine, and dimethylamino moieties in the phenyl ring linked to the thiopyrimidine ring. According to the SAR analysis, thiopyrimidine derivatives with the electron-withdrawing halogen substitutions 5h, 5j, 5a, and 5f

Table 4: Drug-likeness/scores and toxicity calculation of isatin and pyrimidine prototype derivative depends on Osiris property explorer

Component	*TPSA*	*HBA*	*HBD*	*n-ROTB*	*Drug-likeness*	*Drug Score*	*Mutagenicity*	*Tumerogenic*	*Irritation*	*Reproduction action*
5a	100.49	6	0	5	0.89	0.52	green	green	green	green
5b	146.31	9	0	6	-4.20	0.37	green	green	green	green
5c	109.72	7	0	6	0.78	0.56	green	green	green	green
5d	126.51	7	1	5	1.01	0.63	green	green	green	green
5e	103.73	7	0	6	-0.76	0.26	green	red	green	green
5f	100.46	6	0	5	1.63	0.56	green	green	green	green
5g	118.95	8	0	7	2.81	0.62	green	green	green	green
5h	100.49	6	0	5	-2.61	0.29	green	green	green	green
5i	100.49	6	0	5	-2.61	0.29	green	green	green	green
5j	100.49	6	0	5	-0.04	0.50	green	green	green	green

Note: HBA: no. of 'H' bond acceptor; TPSA: area of topological polar surface; HBD: no. of 'H' bond donor; %ABS: percentage of absorption; *n*-ROTB: no. of rotatable bonds.

on the phenyl ring often had stronger anti-tubercular effects than their equivalent counterparts. Furthermore, it was discovered that the location of the substituent has no important effect on the antimicrobial action of analogues since ortho substituted analogues 5h had similar antimicrobial activity to matching para substituted analogues 5i. The substance 5e shown a comparable level of strong action. Out of 10 title components, potent components were found to be (Z)-3-((4-(6-(2-bromophenyl)-2-mercaptopyrimidin -4-yl) phenyl) imino)-1-((dimethylamino) methyl)indolin-2-one 5h, (Z)-3-((4-(6- (4-bromophenyl) -2-mercapto pyrimidin-4-yl)phenyl) imino)-1-((dimethylamino) methyl) indolin-2-one 5i and (Z)-3-((4-(6-(4-dimethylaminophenyl) -2-mercaptopyrimidin-4-yl) phenyl) imino)-1-((dimethylamino) methyl)indolin-2-one 5e.

According to the results of the antimicrobial investigation, compounds with electron withdrawing groups (5b, 5e, 5f, 5j, and 5i) exhibited more antibacterial activity than those with electron giving groups. The location of the group was crucial in determining the activity of compounds containing electron-donating or -withdrawing groups. Antibacterial research showed that an existence of a substituting group at para position of the phenyl ring promotes antimicrobial activity more than at other positions.

CONCLUSION

In conclusion, we synthesized isatin and pyrimidine prototype derivatives and aromatic substitutions by the reaction of (Z)-3-((4-((E)-3-(substitutedphenyl)-3-oxoprop-1-en-1-yl)phenyl) imino)indolin-2-one with 1-chloro-*N,N*-dimethylmethan -amine and thiourea. All newly obtained isatin and pyrimidine prototype derivatives were examined for their anti-tubercular action. From the results it was concluded that the components 5e, 5h and 5j were shown the potent activity against the *M. tuberculosis*. It may be due to presence of halogen atoms and dimethylamino group to the attached phenyl ring. Asp148, Ser94, Gly96, Val189, Phe41, and Ile15 were shown to be the utmost active amino acid remainders in the molecular docking tests, which showed that, substitute group on the mercaptopyrimidine spacer plays a significant role in interaction with the active region of enzyme. These test compounds demonstrated the lowest binding energies with the enoyl-acp reductase enzyme of Mycobacterium TB in *in-silico* investigations. In order to further develop better antitubercular drugs like Mycobacterium TB enoyl-acp reductase inhibitors, the current work gives us knowledge.

REFERENCES

1. Bloom BR, Atun R, Cohen T and Dye C. Tuberculosis. In K. K. Holmes (Eds.) et. al., *Major Infectious Diseases*. (3rd ed.). The International Bank for Reconstruction and Development / The World Bank, 2017.
2. Jeffrey R Starke and Andrea T Cruz. Infectious Diseases of the Fetus and Newborn (Seventh Edition),W.B. Saunders, 201:, 577-600.
3. Prasad R, Gupta N and Banka A. Multidrug-resistant tuberculosis/rifampicin-resistant tuberculosis: Principles of management. Lung India 2018;35(1), 78-81.
4. Vilchèze C, and Jacobs WR. Resistance to Isoniazid and Ethionamide in Mycobacterium tuberculosis: Genes, Mutations, and Causalities. Microbiology *spectrum* 2014; *2*(4), 2013.
5. Smith T, Wolff KA and Nguyen L. Molecular biology of drug resistance in Mycobacterium tuberculosis. Curr Top Microbiol Immunol 2013;374, 53-80.
6. Gengiah TN, Gray AL, Naidoo K and Karim QA. Initiating antiretrovirals during tuberculosis treatment: a drug safety review. Expert Opin Drug Saf 2011;10(4), 559-74
7. Hua Guo. Isatin derivatives and their antibacterial activities. Eur J Med Chem 2019; 164, 678-88.
8. Amandeep Singh, Grant Fong, Jenny Liu, Yun-Hsuan Wu, Kevin Chang, William Park, Jihwan Kim, Christina Tam, Luisa W Cheng, Kirkwood M Land and Vipan Kumar. Synthesis and Preliminary Antimicrobial Analysis of Isatin–Ferrocene and Isatin–Ferrocenyl Chalcone Conjugates. ACS Omega 2018; 3(5), 5808-13
9. Ferraz de Paiva RE, Vieira EG, Rodrigues da Silva D, Wegermann CA and Costa Ferreira AM. Anticancer Compounds Based on Isatin-Derivatives: Strategies to Ameliorate Selectivity and Efficiency. Front Mol Biosci 2021; 7, 627272.
10. Sridhar S, Pandeya S and Clercq E. Synthesis and anti-HIV activity of some isatin derivatives. Boll Chim Farm 2001; 140, 302-5.
11. Raj, Raghu, Biot, Christophe, Kremer, Séverine, Kremer, Laurent, Guérardel, Yann, Gut, Jiri, Rosenthal, Philip Forge, Delphine Kumar and Vipan. 7-Chloroquinoline-isatin Conjugates: Antimalarial, Antitubercular, and Cytotoxic Evaluation. Chemical biology & drug design 2013; 83, 10.
12. Lahari K and Sundararajan R. Design and synthesis of novel isatin derivatives as potent analgesic, anti-inflammatory and antimicrobial agents. J Chem Sci 2020;132, 94.
13. Tawfik NG, Mohamed WR, Mahmoud HS, Alqarni MA, Naguib IA, Fahmy AM and Ahmed OM. Isatin Counteracts Diethylnitrosamine/2-Acetylaminofluorene-Induced Hepatocarcinogenesis in Male Wistar Rats by Upregulating Anti-Inflammatory, Antioxidant, and Detoxification Pathways. Antioxidants 2022; 11, 699.
14. Cheke RS, Firke SD, Patil RR and Bari SB. ISATIN: New Hope Against Convulsion. Cent Nerv Syst Agents Med Chem 2018; 18(2), 76-101.
15. Debnath and Biplab. Synthesis, characterization, and anthelmintic activity of isatin analogs against Pheritima posthuma. Asian J Pharm Clin Res 2015; 8(5), 161-66
16. Jiang D, Wang GQ, Liu X, Zhang Z, Feng LS and Liu *ML*. Isatin Derivatives with Potential Antitubercular Activities. J Heterocyclic Chem 2018; 55, 1263– 79.
17. Mahapatra A, Prasad T and Sharma T. Pyrimidine: a review on anticancer activity with key emphasis on SAR. Futur J Pharm Sci 2021; 7, 123.
18. Wu W, Lan W, Wu C and Fei Q. Synthesis and Antifungal Activity of Pyrimidine Derivatives Containing an Amide Moiety. Front Chem 2021; 9, ID695628.
19. Patel NB, Soni HI, Parmar RB, Chan-Bacab MJ, and Rivera G. 1, 2, 4-triazoles clubbed pyrimidine compounds with synthesis, antimicrobial, antituberculosis, antimalarial, and anti-protozoal studies. Lett Org Chem 2021; *18*(8), 617-24.
20. Rashid Haroon Martines, Marco Antonio Utrera Duarte, Adriana Pereira Jorge, Juliana Rasool, Shagufta Muhammad, Riaz Ahmad, Nasir Umar and Muhammad Naveed. Research developments in the syntheses, anti-inflammatory activities and structure–activity relationships of pyrimidines. RSC Adv 2021; 11(11), 6060-98.
21. Khajeh Dangolani S, Panahi F, Tavaf Z, Nourisefat M, Yousefi

R and Khalafi-Nezhad A. Synthesis and Antioxidant Activity Evaluation of Some Novel Aminocarbonitrile Derivatives Incorporating Carbohydrate Moieties. ACS omega 2018; 3(8), 10341–50.

22. Kostova Irena and Atanasov Petar. Antioxidant Properties of Pyrimidine and Uracil Derivatives. Current Organic Chemistry 2017;21(20), 1-13.
23. Parent AA, Gunther JR, Katzenellenbogen JA. Blocking estrogen signaling after the hormone: pyrimidine-core inhibitors of estrogen receptor-coactivator binding. J Med Chem 2008; 51(20), 6512-30.
24. Esfahanizadeh M, Mohebbi S, Dasht Bozorg B, Amidi S, Gudarzi A, Ayatollahi SA and Kobarfard F. Synthesis and Antiplatelet Aggregation Activity Evaluation of some 2-Aminopyrimidine and 2-Substituted-4,6-diaminopyrimidine Derivatives. Iran J Pharm Res 2015;14(2), 417-27.
25. Iman M, Davood A and Khamesipour A. Design of antimalarial agents based on pyrimidine derivatives as methionine aminopeptidase 1b inhibitor: Molecular docking, quantitative structure activity relationships, and molecular dynamics simulation studies. J Chin Chem Soc 2020; 67, 880– 90.
26. Li S, Yokota T, Wang P, Ten Hoeve J, Ma F, Le TM, Abt ER, Zhou Y, Wu R, Nanthavongdouangsy M, Rodriguez A. Cardiomyocytes disrupt pyrimidine biosynthesis in nonmyocytes to regulate heart repair. The Journal of clinical investigation. 2022 Jan 18;132(2).