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One-Pot Multi-Component Synthesis and Biological Evaluation of Novel Indole-Pyrimidine Derivatives as Potent Anti-Cancer and Anti-Microbial Agents

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Abstract—A series of novel hybrids of indole-pyrimidine moieties were synthesized and evaluated for their in vitro anti-cancer, in vitro anti-bacteria and in vitro anti-fungal activities. The results showed that most of these compounds possessed significant cytotoxic potency against four cancer cell lines, HeLa, HEK 293T and MCF-7. The compounds 4-(3-(benzyloxy)phenyl)-6-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-amine,4-(4-chlorophenyl)-6-(1-methyl-1*H*-indol-3-yl) pyrimidin-2-amine and4-(1*H*-indole-3-yl)-6-phenylpyrimidin-2-amine showed good activity against HEK 293T. The compounds 4-(3-(benzyloxy)phenyl)-6-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-amine and 4-(4-chlorophenyl)-6-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-amine showed good to moderate activity against MCF-7, whereas compound 4-(3-(benzyloxy)phenyl)-6-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-amine exhibit moderate activity against HaLa S3 cell line. The newly synthesized derivatives were also screened for their in vitro anti-bacterial activity against *Bacillus subtilis*, *B. megatherium*, *B. pumilis*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, using streptomycin as a standard drug. Among tested compound 4-(3-(benzyloxy)phenyl)-6-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-amine shows more potent activity compared to standard, where as the remaining analogues exhibited well to moderate activity compared to standard. Anti-fungal screening results suggest that the compound 4-(4-chlorophenyl)-6-(1*H*-indol-3-yl)pyrimidin-2-amineshowed potent activity against *Dreschleria halides*. The remaining compounds showed nearest activity against all the tested fungal strains compared to standard drug.

Keywords: indolo-pyrimidines, claisen-schmidt condensation, one pot synthesis, anti-cancer activity, anti-microbial studies

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INTRODUCTION

The various studies have shown that the impact of cancer is truly shocking in low- or middle-income countries because of fewer resources are available for diagnostics and therapeutic purpose [1]. In recent decades, microtubules have been important molecular targets for the development of anticancer drugs due to their crucial roles in the regulating cancer cell survival and progression. Including cellular signaling, motility, cell shape maintenance, secretion, inter-cellular transport and spindle formation during mitosis [2–5]. Inhibiting tubulin polymerization or interfering with microtubule disassembly ultimately leads to cell cycle arrest orapoptos is of cancer cells [6–8]. Hence, there are commonly two major categories of anti-tubulin agents. Heterocyclic compounds are well-known pharmaceutically active products, and this development of simple and efficient methods of synthesis of compounds incorporating heterocyclic rings has given a new dimension to drug discovery [9]. The indole moiety is probably the most widely spread nitrogen heterocycle in nature. It is an essential part of the amino acid tryptophan and the neurotransmitter serotonin, and the in dolescaf fold is also found in numerous naturally occurring plant based alkaloids. The potential medical pharmacological attention have made them extremely attractive and rewarding research target. The biological activities of indoles cover a wide spectrum, including anti-cancer [10, 11], antimicrobial [12], anti-inflammatory [13], antimalarial [14], cytotoxic [15] and anti-tubercular [16] activities.

The pyrimidine ring system which has a distinguished account starting from its discovery phase as constituent of nucleic acids to their present-day use in chemotherapy [17]. The pyrimidine ring is found in

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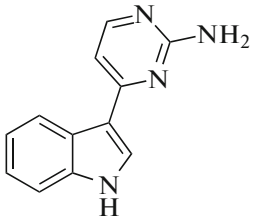
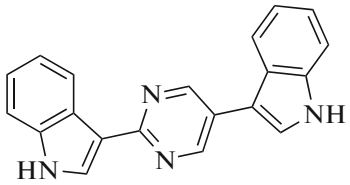
vitamins like thiamine, riboflavin and folic acid [18]. One of the early anti metabolite 5-fluorouracil, a pyrimidine derivative, is used as an anti-neoplastic agent [19]. Over the years, pyrimidine ring systems have been widely explored for their diverse range of biological activities [20–25]. In addition, many pyrimidine analogues have been developed as chemotherapeutic agents [26], anti-leukemic drugs [27], and calcium-sensing receptor antagonists.

As reviewed from earlier literature reports, the indole and pyrimidine analogues to afford novel chemical entities with appreciable biological activity. Such compounds were found to be potent as antitumor [28, 29], antimicrobial [30, 31]and antioxidant [32] agents. Further, some derivatives of meridianins (**I**) and bis-indolo pyrimidine (**II**) (hyrtinadine-A)have show nexcellent inhibition against MCF-7 and HeLa cells [33, 34]. Heterocyclic's possessing free amine group at position-2 of the ring has acquired a unique place in medicinal chemistry. In fact, 2-amino group is a common structural feature in some welle stablishedindolo-pyrimidine drugs [35]. With this in mind, the research has begun to study biologically active properties, especially anti-cancer and anti-bacterial by merging two units of pharmacophore, both an anti-cancer active *N*-methyl indole (Cediranib) scaffold and an anti-bacterial active 2,6-diamine pyrimidine derived from Iclaprim, which are designed and synthesized (Fig. 1). An aromatic substituent at the sixth position of the pyrimidine ring adapts the potency of anti-cancer and anti-bacterial activities. These results and as a part of our research work on the development of novel scaffolds with anti-cancer, antibacterial and anti-fungal activity.



Meridianins (**I**)



Hyrtinadine-A (**II**)

RESULT AND DISCUSSION

The synthesis of indole-pyrimidine derivatives were synthesized by two main steps. Initially the synthesis of 1-methyl-1*H*-indole-3-carbaldehyde obtained by the reaction of with1*H*-indole-3-carbaldehyde and CH_3I in CH_3CN. The second step involves one-pot three components of reaction indole carbaldehyde, substituted acetophenone and guanidine hydrochloride in ethanol to give target indole-pyrimidines in good to high yields. The structure of title compounds and intermediate were established by FT-IR,^{1}H-NMRand Mass spectroscopic analysis.^{1}HNMR spectra of the title compounds (**Va–k**) showed proton of indole-substituted phenyl pyrimidine moiety chemical shift ranging from δ 7.11–8.65 ppm. The free amino proton of the pyrimidine nucleus appeared as a broad singlet in the range at δ 5.23–6.78 ppm. The *N*-methyl protons of indole moiety appeared assinglet in range of δ 3.75–3.83 ppm.

In vitro the anti-cancer activities of the synthesized compounds (**VIa–k**) were evaluated against three different human cancer cell lines HeLa (cervical cancer), MCF-7(breast cancer) and HEK 293T (embryonic kidney) using doxorubicin as standard with the help of MTT assay [36] and the results are summarized Table 1. It was observed that majority of compounds exhibited good to moderate anticancer activity against HeLa (cervical cancer), MCF-7 (breast cancer), and HEK 293T (embryonic kidney) cancer cell lines. Among all compounds, (**Vg**) which contains methyl substituent showed good activity against HEK 293T with IC_{50} values ranging from 66.22 ± 4.26 μg/mL. The analogues (**Vj**), (**Vk**) and (**Va**) bearing 3-benzyloxy, 4-chloro substituent's on aromatic nucleus have shown moderate activity against HEK 293T with IC_{50} values ranging from 67.89 ± 3.38, 67.42 ± 4.20 and 68.65 ± 3.61 μg/mL respectively. On the other hand (**Vj**) and (**Vk**) containing 3-benzyloxy, 4-chloro on aromatic nucleus showed good to moderate activity ranging from 39.03 ± 2.18 to 40.28 ± 3.59 against MCF-7. Whereas compound (**Vj**) exhibit moderate activity ranging of 43.22 ± 3.19 against HeLa with IC_{50} value.

In vitro anti-bacterial activity against en bacteria *viz*, *E. coli*, *B. subtilis*, *B. megatherium*, *B. pumilis*, *P. vulgaris*, *P. mirabilis*, *S. aureus*, *K. pneumonia*, *E. aerogenes*, *S. pyogens*. These results are summarized in Table 2. Streptomycin was taken as positive control. The test compounds and standard drugs are dissolved in DMSO of specific concentrations 600 and 900 μg/mL. Among the tested compounds (**Vj**) shows more potent activity which bearing 3-benzloxy and 3-chloro substituent on aromatic nucleus (**Vd**) exhibit good activity,where as the compound (**Vg**) with methyl substituent on nitrogen of indole nucleus shows equipotent activity against *E. coli* compared to the standard. The compound (**Vc**) and (**Vi**) shows good activity which having a 4-chloroand 4-trifluoromethyl substituent on aromatic nucleus compared to the standard against *B. subtilis*. The introducing of 2-fluoro-4-trifluoromethyl (**Vg**) and a methyl group (**Vh**) on aromatic nucleus more active than the standard drug. Whereas the compound (**Va**) shows nearest activity against *B. pumilis*. The compounds containing 3-chlorosubstituent (**Vd**) and 3-fluoro substituent (**Ve**) showed equipotent activity against *K. pneumonia*. Compounds bearing 4-choro substituent (**Vc**) and 2-fluoro-4-trifluoro methyl substituent's (**Vh**) shows good activity against *E. aerogens* compared to standard drug.

In vitro anti-fungal activity against *Candida albicans*, *Fusarium oxysporium*, *Dreschleria halides* and

Colletotrichum falcatum using itrazole as positive control and results were summarized in Table 3. The compounds **(Vk)**, **(Vd)**, **(Vc)** and **(Vb)** bearing 4-chloro, 3-chloro,4-chloro and 3,4,5-trimethoxy substituent on benzene nucleus shows good activity against all tested fungal strains as compared to standard drug. Moderate to weak activity observed in the case of rest the compounds.

EXPERIMENTAL

All commercially available solvents and reagents were of analytical grade and used without further purification. Melting points were determined on a Gallenkamp melting point apparatus. ^{1}H NMRexperiments were run at 300 MHz on a Bruker Avance NMR spectrometer using TMS as internal standard in DMSO-d_6. Chemical shifts are quoted as δ ppm. The mass spectra were recorded on Jeol SX-102 spectrometer at 70 eV. Column chromatography was performed using silica gel (100–200 mesh size) purchased from Sigma-Aldrich and Thin Layer chromatography was carried out using aluminium sheet pre-coated with silica gel $60F_{254}$ purchased from Merck. IR spectra were obtained using Shimadzu IR-470 spectrometer. CHN analysis was carried out on a Carlo Erba EA 1108 automatic analyzer. Combustion analyzer was found to be within the limits of permissible limits.

General procedure for the synthesis of methyl-1*H*-indole-3-carbaldehyde (II): To a solution of 1*H*-indole-3-carbaldehyde (**I**) (435 mg, 3 mmol) in CH_3CN (30 mL) was added Cs_2CO_3 (829 mg, 6 mmol), and the mixture was refluxed for 2 hours. To this solution, CH_3I (3.3 mmol) was added, and the mixture was heated under reflux for further 1 hour. After the completion of the reaction, the solvent was evaporated under reduced pressure and water was added to the reaction mixture and extracted 3 times for ethyl acetate. The combined organic layers were then dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography with ethylacetate : hexane (1 : 9) to affordmethyl-1*H*-indole-3-carbaldehyde [38] (**II**).

506 mg in 75% yield; mp: 196–198°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ3.03 (s, 3H), 6.44–6.50 (m, 1H), 6.53 (d, 1H, J = 7.0 Hz), 6.61–6.66 (m, 1H), 7.13 (s, 1H), 7.36 (dd, 1H, J = 6.8, 1.5 Hz), 8.99 (s, 1H); MS (ESI, m/z): 160 $[M + H]^+$.

General procedure for the synthesis of compounds (Va–k) (Scheme 1): The mixture of methyl-1*H*-indole-3-carbaldehyde/1*H*-indole-2-carbaldehyde (**II**) (200 mg, 1.37 mmol), acetophenone (**IV**) (0.15 mL, 1.37 mmol), guanidine hydrochloride (**III**) (263 mg, 2.75 mmol) and NaOH (2 mL) in ethanol was allowed to at reflux for 3 hours. After completing the reaction, the reaction mixture was poured into water, and then washed with water thoroughly. The product was filtered, dried and recrystallized from ethanol to afford pure compounds, similar procedure was applied for the rest of compounds.

Table 1. In vitro anti-cancer activityof targeted compounds **(Va–k)**

Compound	IC_{50} values, μg/mL		
	HeLa	MCF-7	HEK 293T
(Va)	63.65 ± 4.19	63.99 ± 3.73	68.65 ± 3.61
(Vb)	67.40 ± 4.03	59.23 ± 3.29	71.84 ± 3.58
(Vc)	57.76 ± 4.28	65.75 ± 3.40	61.00 ± 4.18
(Vd)	59.65 ± 4.17	63.97 ± 3.60	73.34 ± 4.25
(Ve)	55.92 ± 3.10	58.86 ± 3.69	72.63 ± 4.61
(Vf)	59.97 ± 3.48	56.82 ± 4.86	62.47 ± 4.50
(Vg)	55.74 ± 3.27	69.02 ± 3.49	66.22 ± 4.26
(Vh)	48.25 ± 2.95	51.14 ± 3.71	72.78 ± 4.72
(Vi)	56.96 ± 2.85	49.28 ± 2.40	45.74 ± 4.19
(Vj)	43.22 ± 3.19	39.03 ± 2.18	67.89 ± 3.38
(Vk)	51.37 ± 4.73	40.28 ± 3.59	67.42 ± 4.20
Doxorubicin	35.60 ± 1.05	12.76 ± 1.30	65.61 ± 3.27

[a] Each data represents as mean ± SD values. From three different experiments performed in triplicates.
[b] MCF-7: human breast cancer cell line.
[c] HEK 293T: embryonic kidney cancer cell line.
[d] HeLa: human cervical cancer cell line.

4-(1*H*-indol-3-yl)-6-phenylpyrimidin-2-amine (Va): 201 mg in 51% yield: mp: 234–236°C; (FT-IR) (KBr, cm^{-1}): 3544, 1641, 1221, 1175, 1085, 976; ^{1}H NMR (300 MHz, DMSO-d_6): δ6.78 (s, 2H, $-NH_2$), 7.42 (s, 1H, pyrim-H),7.45 (d, 1H, J = 7.28 Hz, indole-H), 7.52 (d, 1H, J = 7.34 Hz, indole-H), 7.63 (t, 1H, indole-H), 7.67 (t, 1H, indole-H), 7.69–7.72 (m, 3H, Ar–H), 7.82(s, 1H, indole-H) 8.10 (d, 2H, J = 8.12 Hz), 9.42 (bs, 1H, indole-NH); MS (ESI, m/z): 287 $[M + H]^+$; Anal. calcd. for $C_{18}H_{14}N_4$: C, 75.50; H, 4.93; N, 19.57. Found: C, 75.48; H, 4.91; N, 19.55.

4-(1*H*-Indol-3-yl)-6-(3,4,5-trimethoxyphenyl)-pyrimidin-2-amine (Vb): 216 mg in 42% yield; mp: 214–216°C; (FT-IR) (KBr, cm^{-1}): 3523, 1617, 1210, 1103, 1009, 910; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 3H, $-OCH_3$), 3.81 (s, 3H, $-OCH_3$), 3.84 (s, 3H, $-OCH_3$), 6.72 (s, 2H, $-NH_2$), 6.92 (s, 1H, –Ar–H), 6.96 (s, 1H, –Ar–H), 7.20 (s, 1H, pyrim-H),7.35 (d, 1H, J = 6.78 Hz, indole-H), 7.45–7.49 (m, 2H, indole-H), 7.60 (d, 1H, J = 6.81 Hz, indole-H), 7.90 (s, 1H, indole-H), 9.83 (bs, 1H, indole-NH); MS (ESI, m/z): 377 $[M + H]^+$; Anal. calcd. for $C_{21}H_{20}N_4O_3$: C, 67.01; H, 5.36; N, 14.88. Found: C, 66.98; H, 5.34; N, 14.85.

4-(4-Chlorophenyl)-6-(1*H*-indol-3-yl)pyrimidin-2-amine (Vc): 230 mg in 52% yield; mp: 221–223°C; (FT-IR) (KBr, cm^{-1}): 3576, 1645, 1242, 1134, 1023, 961; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.65 (s, 2H,

Table 2. In vitro anti-bacterial activity of compounds (**Va–k**)

Compd	μg/mL	Zone of inhibition, mm									
		E. coli	*B. subtilis*	*B. megatherium*	*B. pumilis*	*P. vulgaris*	*P. mirabilis*	*S. aureus*	*K. pneumoniae*	*E. aerogens*	*S. pyogenes*
(Va)	600	1.98	2.12	5.92	**7.42**	4.45	4.32	6.62	3.15	5.19	6.18
	900	3.75	3.80	11.29	**14.80**	8.60	8.49	12.0	6.28	10.28	13.21
(Vb)	600	2.23	6.32	5.56	5.92	2.03	2.65	NA	3.23	3.42	5.32
	900	4.5	12.17	10.63	10.69	3.27	4.29	NA	6.24	6.84	10.24
(Vc)	600	3.26	**6.19**	3.15	6.10	1.12	2.12	5.03	2.13	**6.19**	NA
	900	6.50	**13.12**	6.89	11.20	2.45	4.28	10.2	4.25	**13.45**	NA
(Vd)	600	**6.32**	5.32	5.12	6.32	4.25	6.13	5.92	**6.12**	5.25	5.16
	900	**12.40**	10.80	10.22	13.0	8.46	12.06	11.02	**12.02**	10.28	10.20
(Ve)	600	1.15	NA	5.26	2.26	4.26	5.52	5.69	**5.31**	6.18	5.26
	900	2.78	NA	10.24	5.25	8.55	10.25	11.2	**10.22**	11.25	10.48
(Vf)	600	2.26	5.21	5.52	3.35	4.92	4.95	3.65	3.26	2.25	4.86
	900	4.90	10.2	11.27	6.25	9.04	10.25	7.25	6.29	4.25	9.42
(Vg)	600	**3.36**	5.52	**6.12**	5.21	5.12	5.59	4.56	2.26	6.12	4.26
	900	**6.85**	11.2	**12.06**	10.29	10.11	11.25	8.67	5.45	12.50	8.67
(Vh)	600	1.45	2.36	**6.15**	NA	4.25	4.52	5.68	2.26	**6.91**	3.65
	900	2.24	4.25	**13.45**	NA	8.49	8.25	10.25	4.47	**13.39**	6.47
(Vi)	600	5.52	**6.69**	5.56	2.26	3.36	3.21	5.59	3.36	6.16	2.25
	900	10.02	**13.02**	11.25	4.26	6.80	6.50	11.90	6.28	12.49	4.27
(Vj)	600	**6.96**	3.36	5.26	1.98	5.05	2.98	6.69	5.05	2.45	2.78
	900	**12.9**	6.24	10.28	3.75	10.45	4.00	12.49	10.24	4.47	4.22
(Vk)	600	4.96	2.30	6.22	3.90	5.05	2.98	6.69	5.05	2.45	NA
	900	10.00	3.40	8.18	2.66	9.43	2.00	11.90	11.21	4.47	NA
Std	600	3.06	8.44	6.23	7.25	8.90	12.82	10.34	3.24	4.47	8.91
	900	6.14	16.22	12.80	14.50	16.09	20.34	20.01	6.34	8.76	16.47

Standard: Streptomycin.

Table 3. In vitro anti-fungal activity of compounds (**Va–k**)

Compounds	μg/mL	Zone of inhibition, mm			
		Candida albicans	*Fusarium oxysporium*	*Dreschleria halides*	*Colletotrichum falcatum*
(Va)	600	NA	9.50	2.25	7.05
	900	NA	18.24	5.38	13.25
(Vb)	600	NA	10.25	7.50	**8.05**
	900	NA	20.05	14.25	**15.24**
(Vc)	600	NA	**5.59**	**8.56**	5.62
	900	NA	**10.25**	**16.39**	10.28
(Vd)	600	NA	**7.62**	**7.82**	6.05
	900	NA	**14.25**	**14.54**	11.90
(Ve)	600	6.05	**5.59**	3.82	5.59
	900	12.49	**10.25**	6.59	10.62
(Vf)	600	**5.52**	4.54	5.82	3.45
	900	**10.25**	9.25	11.25	6.42
(Vg)	600	**5.52**	4.26	4.21	2.35
	900	**11.06**	8.28	8.24	4.49
(Vh)	600	5.59	3.32	3.26	3.49
	900	12.50	6.25	6.25	7.25
(Vi)	600	5.25	4.82	8.52	5.26
	900	10.05	8.50	16.25	10.05
(Vj)	600	4.52	5.52	**7.45**	6.92
	900	8.42	10.25	**14.82**	13.24
(Vk)	600	**5.59**	3.32	3.26	3.49
	900	**12.50**	6.25	6.25	7.25
Std	600	5.35	10.80	7.56	9.34
	900	12.01	20.03	14.76	18.26

Stranded: Itrazole.

$-NH_2$), 7.27 (t, 1H, indole-H), 7.32 (t, 1H,indole-H), 7.44 (d, 1H, J = 8.36 Hz, indole-H), 7.52 (d, 1H, J = 8.36 Hz, indole-H), 7.63 (s, 1H, pyrim-H), 7.72 (d, 2H, J = 8.36 Hz, Ar–H); 7.78 (d, 2H, J = 8.36 Hz, Ar–H), 7.98 (s, 1H, indole-H), 9.98 (bs, 1H, indole-NH); MS (ESI, m/z): 321 $[M + H]^+$; Anal. calcd. for $C_{18}H_{13}N_4Cl$: C, 67.40; H, 4.08; N, 17.47. Found: C, 67.39; H, 4.00; N, 17.45.

4-(3-Chlorophenyl)-6-(1*H*-indol-3-yl) pyrimidin-2-amine (Vd): 241 mg in 55% yield; mp: 194–196°C; (FT-IR) (KBr, cm^{-1}): 3558, 1631, 1257, 1187, 1045, 947; ^1H NMR (300 MHz, DMSO-d_6): δ 6.76 (s, 2H, $-NH_2$), 7.37–7.40 (m, 3H, indole-H), 7.46 (d, 1H, J = 8.14 Hz, indole-H), 7.52 (t, 1H, Ar–H), 7.59 (s, 1H, pyrim-H), 7.66–7.71 (m, 2H, Ar–H), 7.76 (d, 1H, J = 8.94 Hz, Ar–H), 7.87 (s, 1H, indole-H), 9.95 (bs, 1H, indole-H); MS (ESI, m/z): 321 $[M + H]^+$; Anal. calcd. for $C_{18}H_{13}N_4Cl$: C, 67.40; H, 4.08; N, 17.47. Found: C, 67.39; H, 4.06; N, 17.46.

4-(3-Fluorophenyl)-6-(1*H*-indol-3-yl) pyrimidin-2-amine (Ve): 211 mg in 50% yield; mp: 252–254°C; (FT-IR) (KBr, cm^{-1}): 3568, 1648, 1267, 1178, 1056, 983; ^1H NMR (300 MHz, DMSO-d_6): δ 6.75 (s, 2H, $-NH_2$), 7.38 (d, 1H, J = 8.57 Hz, indole-H), 7.45 (d, 1H, J = 8.57 Hz, indole-H), 7.56 (t, 2H, indole-H), 7.62 (s, 1H, pyrim-H), 7.76 (s, 1H, Ar–H), 7.82–7.86 (m, 2H, Ar–H), 7.88 (d, 1H, J = 8.96 Hz, Ar–H), 7.93 (s, 1H, indole-H), 10.05 (bs, 1H, –NH); MS (ESI, m/z): 305 $[M + H]^+$; Anal. calcd. for $C_{18}H_{13}N_4F$: C, 71.04; H, 4.31; N, 18.41. Found: C, 71.00; H, 4.29; N, 18.39.

4-(3-(Benzyloxy)phenyl)-6-(1*H*-indol-3-yl)pyrimidin-2-amine (Vf): 229 mg in 42% yield; mp: 261–263°C; (FT-IR) (KBr, cm^{-1}): 3578, 1662, 1740, 1241, 1156, 1012, 945; ^1H NMR (300 MHz, DMSO-d_6): δ 5.24 (s, 2H, $-CH_2$), 6.74 (s, 2H, $-NH_2$), 7.23 (s, 1H, Ar–H), 7.29 (t, 1H, Ar–H), 7.36 (t, 1H, Ar–H), 7.45 (d, 1H, J = 8.12 Hz, Ar–H), 7.47–7.52 (m, 5H, Ar–H), 7.58 (d, 1H, J = 8.24 Hz, indole-H), 7.62 (d, 1H, J = 8.45 Hz, indole-H), 7.65–7.69 (m, 2H,

indole-H), 7.89 (s, 1H, pyrim-H), 8.10 (s, 1H, indole-H), 10.20 (bs, 1H, –NH); MS (ESI, *m/z*): 393 $[M + H]^+$; Anal. calcd. for $C_{25}H_{20}N_4O$: C, 76.51; H, 5.14; N, 14.28. Found: C, 76.50; H, 5.14; N, 14.27.

4-(1-Methyl-1*H*-indol-3-yl)-6-phenylpyrimidin-2-amine (Vg): 240 mg in 64% yield; mp: 218–220°C; (FT-IR) (KBr, cm^{-1}): 3510, 1622, 1216, 1123, 1042, 934; ^1H NMR (300 MHz, DMSO-d_6): δ3.78 (s, 3H, $-CH_3$), 6.59 (s, 2H, $-NH_2$), 7.05 (d, 1H, J = 8.34 Hz, indole-H), 7.10 (d, 1H, J = 8.52 Hz, indole-H), 7.19 (q, 2H, indole-H), 7.30 (d, 1H, J = 8.52 Hz, Ar–H), 7.38 (d, 1H, J = 8.58 Hz, Ar–H), 7.56–7.62 (m, 3H, Ar–H),7.69 (s, 1H, indole-H), 7.81(s, 1H, pyrim-H); MS (ESI, *m/z*): 301 $[M + H]^+$; Anal. calcd. for $C_{19}H_{16}N_4$: C, 75.98; H, 5.37; N, 18.65. Found: C, 75.96; H, 5.34; N, 18.63.

4-(2-Fluoro-4-(trifluoromethyl)phenyl)-6-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-amine(Vh): 248 mg in 51% yield; mp: 266–268°C; (FT-IR) (KBr, cm^{-1}): 3587, 1665, 1245, 1145, 1067, 979; ^1H NMR (300 MHz, DMSO-d_6): δ3.75 (s, 3H, $-CH_3$), 6.72 (s, 2H, $-NH_2$), 7.32 (d, 1H, J = 8.96 Hz, indole-H), 7.43 (d, 1H, J = 8.96 Hz, indole-H), 7.47 (t, 1H, indole-H), 7.52 (t, 1H, indole-H), 7.70 (s, 1H, Ar–H), 7.74 (d, 1H, J = 9.20 Hz, Ar–H), 7.82 (d, 1H, J = 9.20 Hz, Ar–H), 7.92 (s, 1H, pyrim-H), 7.96 (s, 1H, indole-H); MS (ESI, *m/z*): 387 $[M + H]^+$; Anal. calcd. for $C_{20}H_{14}N_4F_4$: C, 62.18; H, 3.65; N, 14.50. Found: C, 62.16; H, 3.63; N, 14.48.

4-(4-(Trifluoromethyl) phenyl)-6-(1-methyl-1*H*-indol-3-yl) pyrimidin-2-amine (Vi): 210 mg in 45% yield; mp: 268–270°C; (FT-IR) (KBr, cm^{-1}): 3564, 1636, 1256, 1123, 1046, 957; ^1H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 3H, $-CH_3$), 6.34 (s, 2H, $-NH_2$), 7.23–7.45 (m, 4H, indole-H), 7.68 (d, 2H, J = 8.56 Hz), 7.73 (d, 2H, J = 8.78 Hz), 7.75 (s, 1H, pyrim-H),7.78 (s, 1H, indole-H); MS (ESI, *m/z*): 369 $[M + H]^+$; Anal. calcd. for $C_{20}H_{15}N_4F_3$: C, 65.21; H, 4.10; N, 15.21. Found: C, 65.19; H, 4.10; N, 15.20.

4-(3-(Benzyloxy)phenyl)-6-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-amine (Vj): 217 mg in 42% yield; mp: 248–250°C; (FT-IR) (KBr, cm^{-1}): 3535, 1645, 1767, 1216, 1120, 1057, 968; ^1H NMR (300 MHz, DMSO-d_6): δ 3.81 (s, 3H, $-CH_3$), 5.18 (s, 2H, $-CH_2$), 5.68 (s, 2H, $-NH_2$), 7.24 (d, 1H, J = 8.56 Hz, indole-H), 7.31 (d, 1H, J = 8.69 Hz, indole-H), 7.36 (t, 2H, indole-H), 7.56 (s, 1H, Ar–H), 7.60 (s, 1H, pyrim-H), 7.63–7.70 (m, 5H, Ar–H), 7.73 (d, 1H, J = 8.34 Hz Ar–H), 7.77 (d, 1H, J = 8.54 Hz, Ar–H), 7.81 (t, 1H, Ar–H), 7.89 (s, 1H, indole-H); MS (ESI, *m/z*): 407 $[M + H]^+$; Anal. calcd. for $C_{26}H_{22}N_4O$: C, 76.83; H, 5.46; N, 13.78. Found: C, 76.83; H, 5.45; N, 13.78.

4-(4-Chlorophenyl)-6-(1-methyl-1*H*-indol-3-yl)-pyrimidin-2-amine (Vk): 236 mg in 56% yield; mp: 254–256°C; (FT-IR) (KBr, cm^{-1}): 3567, 1656, 1235, 1145, 1087, 969; ^1H NMR (300 MHz, DMSO-d_6): δ 3.82 (s, 3H, $-CH_3$), 5.33 (s, 2H, $-NH_2$), 7.34 (d, 1H, J = 8.76 Hz, indole-H), 7.44 (d, 1H, J = 8.82 Hz, indole-H), 7.47(t, 1H, indole-H), 7.54 (t, 1H, indole-H), 7.68 (d, 2H, J = 8.23 Hz, Ar–H), 7.74 (d, 2H, J = 8.33 Hz, Ar–H),7.86 (s, 1H, pyrim-H), 7.92 (s, 1H, indole-H); MS (ESI, *m/z*): 335 $[M + H]^+$; Anal. calcd. for $C_{19}H_{15}N_4Cl$: C, 68.16; H, 4.52; N, 16.73. Found: C, 68.15; H, 4.50; N, 16.73.

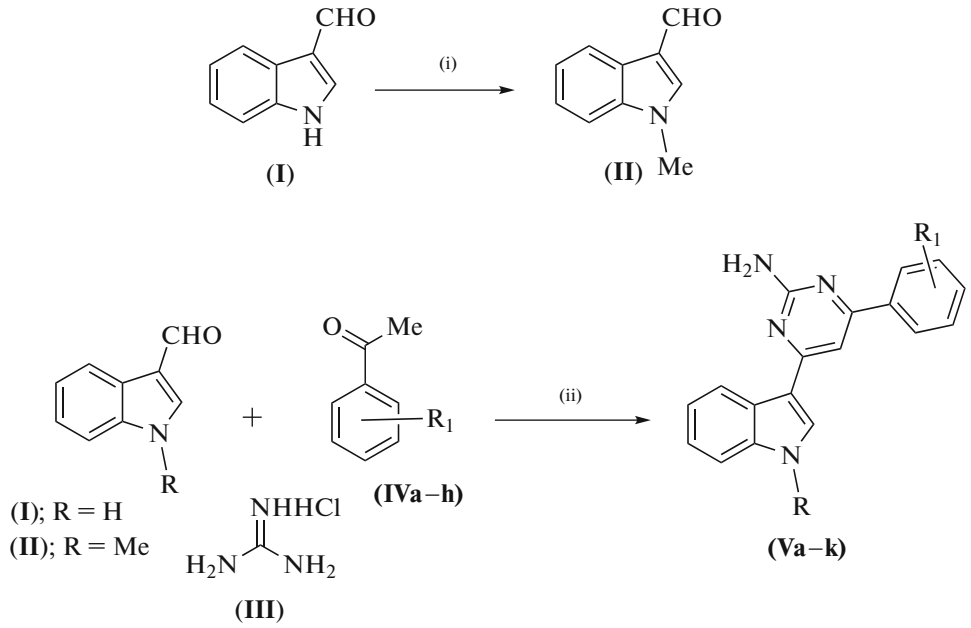
MTT assay: In vitro anti-cancer activity of the synthesized compounds **(Va–k)** was tested using MTT colorimetric assay as per ATCC protocol. Cell lines that were used for testing in vitro cytotoxicity included HeLa, MCF-7 and HEK 293 T was received with job number 1767 from NCCS, Pune. Cell lines were maintained at 37°C in a humidified 5% CO_2 incubator using suitable media prescribed in NCCS Protocol. Decontaminated flasks were incubated for subculture. Cells were passed by 12 numbers. After getting 70% confluence; from culture flasks take 100 μL cell suspension and make a cell count using haemocytometer and found 5000–6000 per well in a 96-well plate. Cell suspension was mixed thoroughly by pippetting several times to get a uniform single cell suspension. Different dilutions of drugs solutions 3, 10, 30, 100 μg/mL were made in media with final 0.5% DMSO. 100 μL of cell suspension was transferred aseptically to each well of a 96 well plate and to it 100 μL of drug solution in (quadruplicate) in media was added. The plate was then incubated at 37°C for 72 hours in CO_2 incubator. After 48 hours of incubation, 20 μL of MTT was added to each well. The plate was again incubated for 2 hours, 80 μL of analysis buffer was added to each well the plate was wrapped in aluminum foil to prevent the oxidation of the dye and the plate was placed on a shaker for 30 minutes. The absorbance were recorded on the ELISA reader (Biotech EL×800) at 570 nm wavelength. We will calculate % inhibition by following formula.

% inhibition = Control ODs-Test ODs+Control ODs× 100 and finally IC_{50} Values to asses anti-cancer activity. Doxorubicin was used as the standard drug in the assay.

Antimicrobial activity: Antimicrobial activities of compounds **(Va–k)** were carried out by agar well diffusion [37] method against test organisms. Using the sterile cork borer, wells 6 mm were made in to each Petri-plate. DMSO used as a negative control. The test compounds and standard drugs are dissolved in DMSO of specific concentrations 600 and 900 μg/mL test compounds are filled in the wells and incubated at 37°C and the diameter of the inhibition zones were measured after 24 hours in case of bacteria and after 48 hours in case of fungi. After appropriate incubation, the diameter of zone of inhibition of each well was measured. Ciprofloxacin was used as reference drug for antibacterial activity agent. Itrazole drug was used as reference for anti fungal agent. DMSO used as a negative control.Duplicate were maintained and the average values were calculated for eventually antibacterial and antifungal activity.

CONCLUSIONS

The presented work described an efficient synthesis of new indole-pyrimidine (**Va–k**) derivatives by applying a multi-component one-pot method. The target molecules investigated for their in vitro cytotoxicity and anti-microbial properties by MTT assay and broth dilution method [37] respectively. The most susceptible cell lines comprised of HeLa, HEK 293T and MCF-7 with majority of compounds showing higher inhibition against these cell lines. The compounds (**Vj**) and (**Vk**) showed good to moderate activity against MCF-7, whereas compound (**Vj**) exhibit moderate activity against HaLa cell line. Whereas the compound (**Vg**) showed potent activity on HEK 293T and the remaining compounds (**Vj**), (**Vk**) and (**Va**) exhibited moderate activity against HEK 293T. The other hand, in case of anti-bacterial studies, compound (**Vj**) shows more potent activity compared to standard, where as the remaining analogues (**Vg**), (**Vc**), (**Vh**), (**Vi**), (**Va**), (**Ve**) and (**Vk**) exhibited well to moderate activity compared to standard. Anti-fungal screening results suggest that the compounds (**Vk**), (**Vd**), (**Vc**) and (**Vb**) showed potent activity. They may be considered as a promising leads for future design of potent and selective antimicrobial agents.



(**IVa**); R_1 = H

(**IVb**); R_1 = 3,4,5-trimethoxy

(**IVc**); R_1 = 4-chloro

(**IVd**); R_1 = 3-chloro

(**IVe**); R_1 = 3-fluoro

(**IVf**); R_1 = 3-benzyloxy

(**IVg**); R_1 = 2-fluoro-4-trifluoromethyl

(**IVh**); R_1 = 4-trifluoromethyl

(**Va**); R,R_1 = H

(**Vb**); R = H, R_1 = 3,4,5-trimethoxy

(**Vc**); R = H, R_1 = 4-chloro

(**Vd**); R = H, R_1 = 3-chloro

(**Ve**); R = H, R_1 = 3-fluoro

(**Vf**); R = H, R_1 = 3-benzyloxy

(**Vg**); R = Me, R_1 = H

(**Vh**); R = Me, R_1 = 2-fluoro-4-trifluoromethyl

(**Vi**); R = Me, R_1 = 4-trifluoromethyl

(**Vj**); R = Me, R_1 = 3-benzyloxy

(**Vk**); R = Me, R_1 = 4-chlolo

Scheme 1. Reagents and conditions: (i) CH_3I, Cs_2CO_3, CH_3CN, reflux, 3 h. (ii) EtOH, aq. NaOH, reflux, 3 h.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving animals or human participants performed by any of the authors.

Conflict of Interests

The authors declare that they have no conflict of interest

REFERENCES

1. Unger-Saldaña, K., *World. J. Clin. Oncol.,* 2014, vol. 5, pp. 465–477. https://doi.org/10.5306/wjco.v5.i3.465
2. Mora-Bermúdez, F. and Huttner, W.B., *Mol. Biol. Cell.,* 2015, vol. 26, pp. 4302–4306. https://doi.org/10.1091/mbc.E15-03-0152
3. Kaur, R., Kaur, G., Gill, R.K., Soni, R., and Bariwal, J., *Eur. J. Med. Chem.,* 2014, vol. 87, pp. 89–124. https://doi.org/10.1016/j.ejmech.2014.09.051
4. Driowya, M., Leclercq, M.J., Verones, V., Barczyk, A.M., Lecoeur, N., Renault, N., Flouquet, A., Ghinet P., and Berthelot, N., *Eur. J. Med. Chem*., 2016, vol. 115, pp. 393–405. https://doi.org/10.1016/j.ejmech.2016.03.056
5. Z. Gan-Or, G.A., Rouleau, E.E., and Benarroch, *Neurology*, 2016, vol. 87, pp. 2173–2184. https://doi.org/10.1212/wnl.0000000000003444
6. Kumar, A.S., Reddy, M.A., Jain, N., Kishor, C., Murthy, T.R., Ramesh, B.D., Supriya, B., Addlagatta, A., Kalivendi, S.V., and Sreedhar, B., *Eur. J. Med. Chem*., 2013, vol. 60, pp. 305–324. https://doi.org/10.1016/j.ejmech.2012.12.008
7. Strzyz, P., *Nat. Rev. Mol. Cell Biol.,* 2016, vol. 17, pp. 333–341. https://doi.org/10.1038/ nrm.2016.63
8. Dumontet, C. and Jordan, M.A., *Nat. Rev. Drug Discov.,* 2010, vol. 9, pp. 790–803. https://doi.org/10.1038/nrd3253
9. Shelke, S.H., Mhaske, P.C., Kasam, S.K., and Bobade, V.D., *J. Heterocycl. Chem*., 2014, vol. 51, pp. 1893–1897. https://doi.org/10.1002/jhet.1910
10. Singh, P., *Eur. J. Med. Chem.,* 2014, vol. 74, pp. 440–450. https://doi.org/10.1016/j.ejmech.2013.12.047
11. Sharma, S.K., Kumar, P., Narasimhan, B., Ramasamy, K., Mani, V., Mishra, K.R., Majeed, A., *Eur. J. Med. Chem.,* 2012, vol. 48, pp. 16–25. https://doi.org/10.1016/j.ejmech.2011.11.028
12. Mehndiratta, S., Hsieh, Y.L., Liu, Y.M., and Wang, A.W., *Eur. J. Med. Chem.,* 2014, vol. 85, pp. 468–479. https://doi.org/10.1016/j.ejmech.2014.08.020
13. Liew, L.P.P., Fleming, J., Longeon, A., Mouray, E., and Florent, I., *Tetrahedron,* 2014, vol. 70, pp. 4910–4920. https://doi.org/10.1016/j.tet.2014.05.068
14. Yamuna, E., Kumar, R.A., and Zeller, M., *Eur. J. Med. Chem.,* 2012, vol. 47, pp. 228–238. https://doi.org/10.1016/j.ejmech.2011.10.046
15. Comín-Anduix, B., Agell, N., Bachs, O., and Ovadi, J., *Mol. Pharmacol.,* 2001, vol. 60, pp. 1235–1242. https://doi.org/10.1124/mol.60.6.1235
16. Nassar, E., *J. Am. Sci.,* 2010, vol. 6, pp. 463–471. https://doi.org/10.1134/S0003683810050017
17. Nagarapu, L., Vanaparthi, S., Bantu, R., and Kumar, C.G., *Eur. J. Med. Chem.,* 2013, vol. 69, pp. 817–822. https://doi.org/10.1016/j.ejmech.2013.08.024
18. Pingxian Liu, Yang Yang, Yunxiang Tang, Tao Yang, Zitai Sang, and Zhiyong Liu, *Eur. J. Med. Chem.,* 2019, vol. 163, pp. 169–182. https://doi.org/10.1016/j.ejmech.2018.11.054
19. Ballell, A.F., Robert, G.A.C., and Chung Young, R., *Bioorg. Med. Chem. Lett*., 2007, vol. 17, pp. 1736–1740. https://doi.org/10.1016/j.bmcl.2006.12.066
20. Gorlitzer, K., Herbig, S., and Walter, R.D., *Org. Chem. Int.*, 1997, vol. 52, pp. 670–672. https://doi.org/10.1155/2013/582079
21. Malik, V., Singh, P., and Kumar, S., *Tetrahedron,* 2006, vol. 62, pp. 5944–5951. https://doi.org/10.1016/j.tet.2006.04.018
22. Al-Issa, A.S., *Saudi Pharm. J.*, 2013, vol. 21, pp. 305–316. https://doi.org/10.1016/j.jsps.2012.09.002
23. Wagner, E., Al-Kadasi, K., Zimecki, M., and Sawka-Dobrowolska, W., *Eur. J. Med. Chem*., 2008, vol. 43, pp. 2498–2504. https://doi.org/10.1016/j.ejmech.2008.01.035
24. Miyazaki, Y., Matsunaga, S., Tang J., Maeda, Y., Nakano, M., and Philippe, R.J., *Bioorg. Med. Chem. Lett.,* 2005, vol. 15, pp. 2203–2207. https://doi.org/10.1016/j.bmcl.2005.03.034
25. Christopherson, R.I. and Lyons, S.D., *Med. Res. Rev.,* 1990, vol. 10, pp. 505–548. https://doi.org/10.1002/med.2610100406
26. Raffa, D., Daidone, G., Maggio, B., and Cascioferro S., *II Farmaco Sci.,* 2004, vol. 59, pp.451–455. https://doi.org/10.1016/j.farmac.2003.10.006
27. Yang, W., Ruan, Z., Wang, Y., Van-Kirk, K., Ma, Z., and Arey, B.J., *J. Med. Chem.,* 2009, vol. 52, pp. 1204–1208. https://doi.org/10.1021/jm801178c
28. Hanselmann, R., Job, G.E., Johnson, G., Lou, R.L., and Martynow, J.G., *Org. Process Res. Dev.,* 2010, vol. 14, p. 152. https://doi.org/10.1021/op900252a
29. Nikhila, G., Dayakumar, D., and Manjunatha Kumsi., *J. Saudi Chem. Soc.,* 2017, vol. 7, pp. 761–775. https://doi.org/10.1016/j.jscs.2015.09.003

30. Biradar, J.S. and Somappa, S.B., *Pharm. Lett.,* 2012, vol. 4, pp. 344–348. http://scholarsresearchlibrary.com/archive.html

31. Tanveer Mahamad Alli, S. and Habtamu D., *J. Chem.,* 2020, vol. 2020, pp. 1–9. https://doi.org/10.1155/2020/4358453

32. Mohamed, M.S., Youns, M.M., and Ahmed, N.M., *Med. Chem. Res.,* 2014, vol. 23, pp. 3374–3388. https://doi.org/10.1007/s00044-014-0916-1

33. Akue-Gedu, R., Debiton, E., Ferandin, Y., and Meijer, L.M., *Bioorg. Med. Chem.,* 2009, vol. 17, pp. 4420–4424. https://doi.org/10.1016/j.bmc.2009.05.017

34. Radwan, M.A. and El-Sherbiny, M., *Bioorg. Med. Chem.,* 2007, vol. 15, pp. 1206–1211. https://doi.org/10.1016/j.bmc.2006.11.023

35. Selvam, T.P., James, C.R., Dniandev, P.V., and Valzita, S.K., *Res. Pharm.*, 2012, vol. 2, pp. 01–09. https://doi.org/10.24959/ophcj.14.804

36. Mosmann, T., *J., Immunol. Methods,* 1983, vol. 65, pp. 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

37. National Committee for Clinical Laboratory, *Nat. Commun. Clin. Lab. Stands.*, 1982, vol. 33, p. 242.

38. Romagnoli, R., Prencipe, F., Lopez-Cara, L.C., and Oliva, P., *J. Enzyme Inhib. Med. Chem.*, 2018, vol. 33, pp. 727–742. https://doi.org/10.1080/14756366.2018.1450749