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Synthesis, characterization, and screening for the antimicrobial activity of 2-(3-(2-cyano-2-(*p*-tolylamino)vinyl)-1*H*-indol-1-yl)-N-arylacetamide derivatives

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Herein is described an account of research work that focuses on synthesis of some novel α,β-unsaturated compounds. To afford 2-(3-(2-cyano-2-(*p*-tolylamino)vinyl)-1*H*-indol-1-yl)-N-arylacetamide derivatives, Knoevenagel condensation of 2-(3-formyl-1*H*-indol-1-yl)-N-arylacetamides and 2-cyano-N-(4-methylphenyl) acetamide have been carried out. 2-(3-Formyl-1*H*-indol-1-yl)-N-arylacetamides have been easily derived by employing indole-3-carbaldehyde with 2-chloro-N-arylacetamides. All the synthesized compounds have been characterized using analytical techniques such as mass spectrometry, Fourier transform infrared (FT-IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy. The biological activity of all the synthesized compounds have been evaluated against a series of bacterial and fungal strains (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Rhizopus oryzae*, and *Aspergillus parasiticus*). Among the synthesized compounds **5e**, **5g** and **5h** demonstrate excellent to moderate antibacterial activity when compared to standard drugs such as ampicillin and streptomycin. Whereas **5e** exhibits antifungal property as compared to standard drug nystatin.

Keywords: Indole-3-carbaldehyde, Knoevenagel condensation, α, β-Unsaturated compounds, Antimicrobial

Heterocyclic compounds[1] are general fragments of various active pharmaceutical ingredients. It exhibits remarkable characteristics when applied in medicinal contexts, and numerous among them can be regarded as privileged scaffolds. The utilization of diverse heterocyclic compounds in various medications[2] is linked to the progress achieved in the synthesis methodologies of heterocycles as it plays a crucial role in the pharmaceutical industry. Hence, these compounds hold significance for chemists as they aid in the discovery of novel compounds for potential drug applications.

The study reveals that a vast array of nitrogen-containing heterocycles[3] exhibits significant biological activity. Among the various nitrogen containing heterocycles, indole and its derivatives are ubiquitous in nature, serving as a key component in the structures of numerous natural products. Indole-containing compounds presents extraordinary medicinal activity such as antimicrobial[4], anti-inflammatory[5], anticancer[6], and antioxidant[7], *etc*.

The Knoevenagel condensation[8,9] involving indole-3-carbaldehyde and active methylene compounds leads to the formation of α, β-unsaturated derivatives of indole, which exhibit significant biological and pharmaceutical activities. The present work is related to Knoevenagel condensation between 2-(3-formyl-1*H*-indol-1-yl)-N-arylacetamide derivatives and 2-cyano-N-(4-methylphenyl)acetamide to yield a set of compounds demonstrating antimicrobial activity that is equally potent when compared to established standard drugs like ampicillin, nystatin, and streptomycin.

Results and Discussion

Chemistry

Synthesis of 2-(3-(2-cyano-2-(*p*-tolylamino)vinyl)-1*H*-indol-1-yl)-N-arylacetamide derivatives (**5a-h**) involves 4 steps (Fig. 1, Scheme 1). In First step indole-3-carbaldehyde (**2**) was synthesized by Vilsmeier–Haack reaction of indole (**1**) with DMF and $POCl_3$ at 0-5°C. Step 2 employs synthesis of 2-chloro-N-arylacetamides (**3a-h**) derivatives from 2-chloroacetylchloride and substituted anilines in acetone as a solvent. While in third step 2-(3-formyl-1H-indol-1-yl)-N-arylacetamide derivatives (**4a-h**) were synthesized by combining 2-chloro-N-arylacetamides (**3a-h**) and indole-3-carbaldehyde (**2**)

in DMF with sodium hydride (NaH) as a base under reflux conditions. Final step involves Knoeven agel condensation of 2-(3-formyl-1H-indol-1-yl) -N-arylacetamide derivatives (**4a-h**, Table 1) and 2-cyano-N-(4-methylphenyl)acetamide in methanol

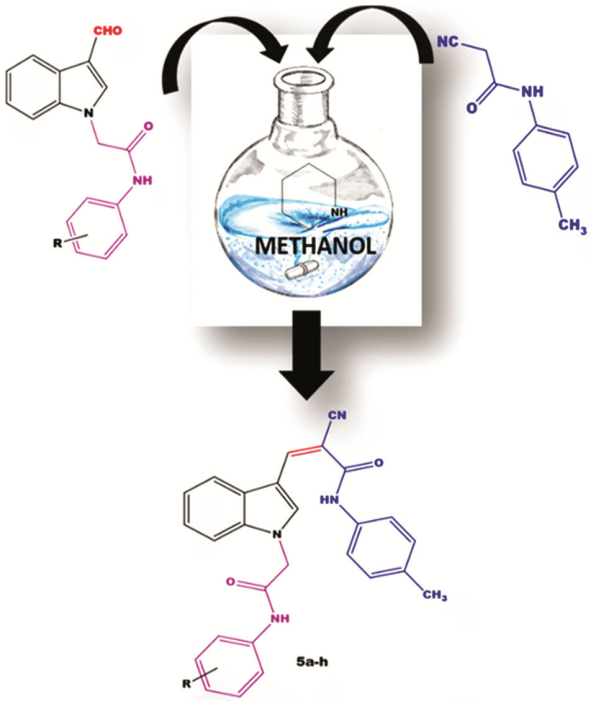
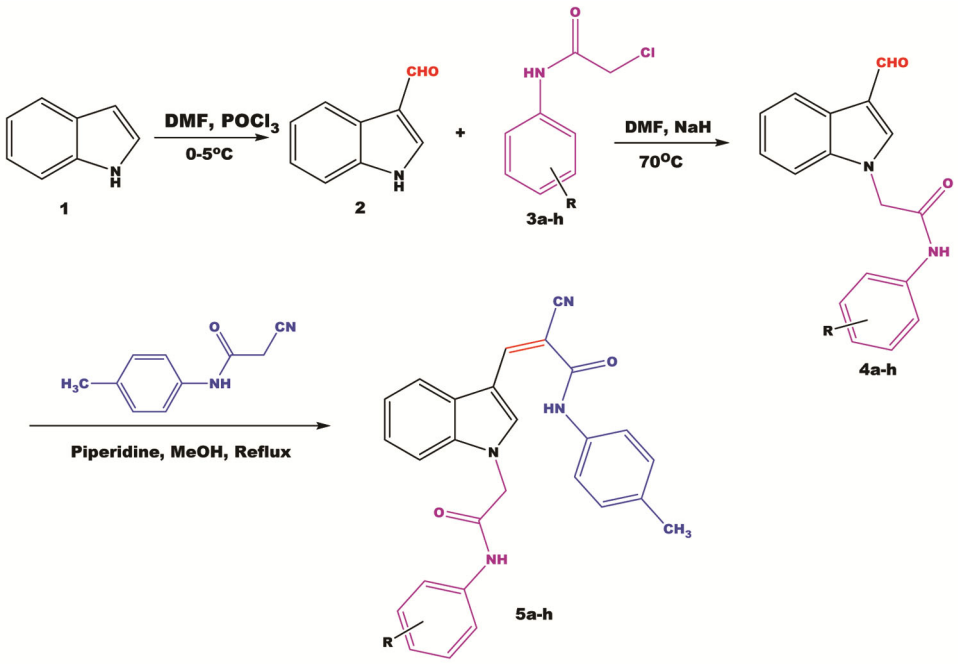


Fig. 1 — Synthesis of 2-(3-(2-cyano-2-(*p*-tolylamino)vinyl)-1*H*-indol-1-yl)-N-arylacetamide derivatives **5a-h** in 4 steps

using piperidine as catalyst. The reaction proceed by nucleophilic addition of aldehyde group of 2-(3-formyl-1H-indol-1-yl)-N-arylacetamide to the active methylene group of 2-cyano-N-(4-methylphenyl) acetamide followed by dehydration reaction to give 2-(3-(2-cyano-2-(*p*-tolylamino)vinyl)-1*H*-indol-1-yl)-N-arylacetamide derivatives (**5a-h**, Table 2).

Antimicrobial Activity

The compounds of the present work were screened for the antibacterial [10-16] and antifungal[17,18] activity using 100 ppm concentration in DMF by cup and well method for gram-positive bacteria and fungi, while 50 ppm concentration for gram-negative bacteria. The micro-organisms *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* were used as bacterial strains and *Rhizopus oryzae* and *Aspergillus parasiticus* were used as fungal strains. The antibiotics used for activity were streptomycin (antibacterial against gram negative bacteria), ampicillin (antibacterial against gram positive bacteria), nystatin (antifungal) and the dilutions of the compounds were made 1000, 500, 250, 125 and 62.5 (in ppm). The findings are as displayed in Table 3. Among the range of compounds **5a-h**, it was analysed that **5g** and **5h** possess a broad-spectrum antibacterial efficacy and forbids the growth of both gram-negative and gram-positive bacteria. Furthermore, compounds **5e**, **5g**, and **5h** show



Scheme 1 — Synthetic scheme for the preparation of compounds **5a-h**

Table 1 — Mass spectral analysis of 2-(3-formyl-1*H*-indol-1-yl)-N-arylacetamide derivatives **4a-h**

Compd	-R	Mol. Formula	Yield (%)	Mol. Mass (*m/z*)
4a	4-H	$C_{17}H_{14}N_2O_2$	85	278.15
4b	4-Me	$C_{18}H_{16}N_2O_2$	81	292.50
4c	3-Cl	$C_{17}H_{13}ClN_2O_2$	78	312.20
4d	4-F	$C_{17}H_{13}FN_2O_2$	82	296.25
4e	2-Me	$C_{18}H_{16}N_2O_2$	84	292.20
4f	4-Cl	$C_{17}H_{13}ClN_2O_2$	73	312.00
4g	3-NO_2	$C_{17}H_{13}N_3O_4$	71	323.15
4h	4-OMe	$C_{18}H_{16}N_2O_3$	65	308.12

Table 2 — Physical characterization data of 2-(3-(2-cyano-2-(*p*-tolylamino)vinyl)-1*H*-indol-1-yl)-N-arylacetamide derivatives

Compd	-R	Mol. Formula	Colour	Yield (%)	m.p. (□)
5a	4-H	$C_{27}H_{22}N_4O_2$	Light yellow	82	270
5b	4-Me	$C_{28}H_{24}N_4O_2$	Yellow	78	282
5c	3-Cl	$C_{27}H_{21}ClN_4O_2$	Yellow	72	286
5d	4-F	$C_{27}H_{21}FN_4O_2$	Light yellow	75	298
5e	2-Me	$C_{28}H_{24}N_4O_2$	Yellow	84	302
5f	4-Cl	$C_{27}H_{21}ClN_4O_2$	Yellow	74	290
5g	3-NO_2	$C_{27}H_{21}N_5O_4$	Dark Yellow	70	280
5h	4-OMe	$C_{28}H_{24}N_4O_3$	Light yellow	79	280

Table 3 — Antimicrobial activity data of 2-(3-(2-cyano-2-(*p*-tolylamino)vinyl)-1*H*-indol-1-yl)-N-arylacetamide derivatives

	Antibacterial MIC (μg mL^{-1})				Antifungal MIC (μg mL^{-1})	
Compd	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E.coli*	*A. parasiticus*	*R. oryzae*
Ampicillin	100	100	–	–	–	–
Streptomycin	–	–	50	50	–	–
Nystatin	–	–	–	–	100	100
5a	1000	1000	500	500	500	500
5b	1000	1000	1000	1000	250	250
5c	1000	1000	500	500	1000	1000
5d	1000	1000	500	500	1000	1000
5e	1000	1000	125	125	125	125
5f	1000	1000	500	500	500	500
5g	125	125	125	125	1000	1000
5h	250	250	250	250	1000	1000

potential activity against gram-negative bacteria. In contrast, compounds **5b** and **5e** primarily demonstrated antifungal activity (Fig. 2).

Experimental Section

Synthesis of 1*H*-indole-3-carbaldehyde, 2

50 mL of DMF was taken in a round-bottom flask, and 20 mL of phosphorus oxychloride (160 mmol) was slowly added over 30 minutes at 0-5°C. The formylation mixture was brought up to RT and solution of indole (220 mmol) in DMF was added slowly. After completion of the reaction the mixture was gushed into ice shavings which yielded a pellucid red colored solution. To this 100 mL 6N sodium hydroxide solution was added till light brown colored solid particles were separated. The solid product was filtered, washed with chilled water and purified by recrystallization from ethanol[19]. Yield 87%. Light brown crystals. m.p.196°–198°C

Synthesis of 2-chloro-N-arylacetamide derivatives, 3a-h

Various substituted anilines (10 mmol) and 2-chloroacetyl chloride (10 mmol) were dissolved in acetone and stirred at RT for approximately 30 min using a round-bottom flask (RBF). The reaction was traced using TLC. Upon completion of the reaction, the 2-chloro-*N*-arylacetamide derivatives were

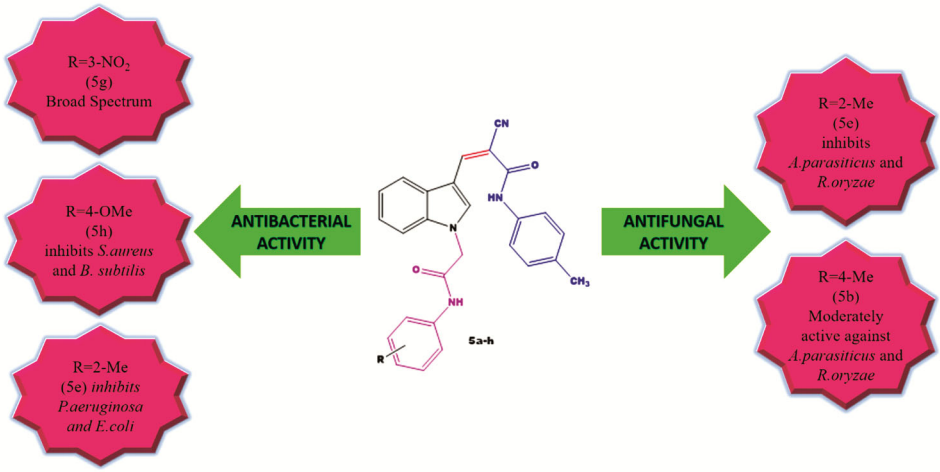


Fig. 2 — Structural activity relationship due to different functional groups

discharged into ice shavings, and it was filtered, dried, and purified by recrystallization from methanol[20].

General procedure for synthesis of 2-(3-formyl-1*H*-indol-1-yl)-N-arylacetamide derivatives, 4a-h

The solution of indole-3-carbaldehyde **2** (10 mmol) in DMF (10 mL) and excess sodium hydride (12 mmol) were stirred for 1-2 h at RT in an RBF. To this reaction mixture 2-chloro-N-arylacetamide derivatives **3a-h** (11 mmol) were added. The reaction mixture was heated at 60-70°C temperature overnight. The progress of reaction was traced by thin-layer chromatography (TLC). Once the reaction was complete, it was allowed to cool to ambient temperature and was poured into crushed ice. The resulting solid products were isolated using filtration and subsequently dried[21].

General procedure for synthesis of 2-(3-(2-cyano-2-(*p*-tolylamino)vinyl)-1*H*-indol-1-yl)-N-arylacetamide derivatives, 5a-h

A solution containing 2-(3-formyl-1*H*-indol-1-yl)-N-arylacetamide derivatives **4a-h** (10 mmol) in 30 mL of methanol was subjected to reflux at a temperature range of 60-70°C, along with 2-cyano-N-(4-methylphenyl)acetamide (10 mmol). A small amount of piperidine was added as a catalyst to it. The progress of the reaction was supervised by TLC. The reaction mixture was cooled up to RT and poured into chilled water (200 mL). The solid product so formed was collected by filtration and purified by recrystallization from methanol[22].

Spectral Data

2-Cyano-3-(1-(2-oxo-2-(phenylamino)ethyl)-1*H*-indol-3-yl)-N-(*p*-tolyl)acrylamide, 5a: Light yellow solid. IR (KBr): 3055(C-H str., Ar-H), 2931, 2800(C-H str.Ali., CH_2), 2214(-CN str.), 1890(-$CONH_2$ str.), 1658, 1527, 1404 cm^{-1} (Ar C=C bend.); ^{1}H NMR (DMSO-d_6): δ 10.5(s, 1H,-NH-amide), 10.1 (s, 1H,-NH-amide), 8.6(d, 2H, -C-H), 8.03-7.07(m, 13H, ArH), 5.35(s, 2H, Indole-N-CH_2-), 2.30(s, 3H, -CH_3); MS: *m/z* 434.

2-Cyano-3-(1-(2-oxo-2-(*p*-tolylamino)ethyl)-1*H*-indol-3-yl)-N-(*p*-tolyl)acrylamide, 5b: Yellow solid. IR (KBr): 3348, 3271, 3117, 3047(C-H str., Ar-H), 2924, 2407(C-H str. Ali., CH_2), 2206(-CN str.), 1882(-$CONH_2$ str.), 1666,1589,1519,1404 cm^{-1} (Ar C=C bend.); MS: *m/z* 448.5.

3-(1-(2-((3-Chlorophenyl)amino)-2-oxoethyl)-1*H*-indol-3-yl)-2-cyano-N-(*p*-tolyl)acrylamide,5c: Yellow solid. IR (KBr): 3117, 3055(C-H str., Ar-H), 2924(C-H str. Ali., -CH_2), 2206(-CN str.), 1874 (-$CONH_2$ str.), 1674, 1411 cm^{-1} (Ar C=C bend.); ^{1}H NMR (DMSO-d_6): δ 10.7(s, 1H,-NH-amide), 10.1(s, 1H,-NH-amide), 8.6(d, 2H, -C-H), 8.05(d, 1H-C-H), 8.04-7.1(m, 11H, ArH), 5.37(s, 2H, Indole-N-CH_2-), 2.03(s, 3H, -CH_3); MS: *m/z* 468.2.

2-Cyano-3-(1-(2-((4-fluorophenyl)amino)-2-oxoethyl)-1*H*-indol-3-yl)-N-(*p*-tolyl)acrylamide, 5d: Light yellow solid. IR (KBr): 3055(C-H str., Ar-H), 2931, 2800(C-H str. Ali., CH_2), 2260(-CN str.), 1890(-$CONH_2$ str.), 1666, 1519, 1414 cm^{-1} (Ar C=C bend.); ^{1}H NMR (DMSO-d_6): δ 10.5(s, 1H,-NH-amide), 10.09(s, 1H,-NH-amide), 8.6(d, 1H, -C-H), 8.05(d, 1H,-C-H), 8.03-7.16(m, 12H, ArH), 5.34(s, 2H, Indole-N-CH_2-), 2.33(s, 3H, -CH_3); MS: *m/z* 451.9.

2-Cyano-3-(1-(2-oxo-2-(*o*-tolylamino)ethyl)-1*H*-indol-3-yl)-N-(*p*-tolyl)acrylamide, 5e: Yellow solid. IR (KBr): 3039(C-H str., Ar-H), 2924, 2862(C-H str.Ali., CH_2), 2206(-CN str.), 1890(-$CONH_2$ str.), 1666, 1581, 1519, 1404 cm^{-1} (Ar C=C bend.); ^{1}H NMR (DMSO-d_6): δ 10.08(s, 1H,-NH-amide), 9.85(s, 1H,-NH-amide), 8.6(d, 1H, -C-H), 8.58 (d, 1H,-C-H), 8.05-7.09(m, 12H, ArH), 5.39(s, 2H, Indole-N-CH_2-), 2.30(s, 3H, -CH_3); MS: *m/z* 448.9.

3-(1-(2-((4-Chlorophenyl)amino)-2-oxoethyl)-1*H*-indol-3-yl)-2-cyano-N-(*p*-tolyl)acrylamide, 5f: Yellow solid. IR (KBr): 3271, 3117, 3055(C-H str., Ar-H), 2916(C-H str. Ali., -CH_2), 2206(-CN str.), 1882 (-$CONH_2$ str.), 1674, 1590, 1519, 1404 cm^{-1} (Ar C=C bend.); ^{1}H NMR (DMSO-d_6): δ 10.67 (s, 1H,-NH-amide), 10.12(s, 1H,-NH-amide), 8.60(d, 1H, =C-H), 8.58(d, 1H,-C-H), 8.03-7.17(m, 12H, ArH), 5.36 (s, 2H, Indole-N-CH_2-), 2.30(s, 3H, -CH_3); MS: *m/z* 468.3.

2-Cyano-3-(1-(2-((3-nitrophenyl)amino)-2-oxoethyl)-1*H*-indol-3-yl)-N-(*p*-tolyl)acrylamide, 5g: Dark yellow solid. IR (KBr): 3317, 3117(C-H str., Ar-H), 2924, 2785(C-H str. Ali.- CH_2), 2198(-CN str.), 1882(-$CONH_2$ str.), 1689, 1589, 1527, 1342 cm^{-1} (Ar C=C bend.); ^{1}H NMR (DMSO-d_6): δ 11.007(s, 1H, -NH-amide), 10.095(s, 1H,-NH-amide), 8.59-8.63(t, 2H, -C-H), 8.05(t, 1H,-C-H), 8.03-7.17(m, 11H, ArH), 5.40(s, 2H, Indole-N-CH_2-), 2.30(s, 3H, -CH_3); MS: *m/z* 479.1

Conclusion

The aim of this current research was to synthesise, analyse, and explore the antimicrobial activity of 2-(3-(2-cyano-2-(*p*-tolylamino)vinyl)-1*H*-indol-1-yl)-N-arylacetamide derivatives. The results of antimicrobial study shows that compound **5g** and **5h** is found to be equipotent to standard drug ampicillin (MIC = 100 μg mL^{-1}) against gram-positive bacteria *S. aureus* and *B. subtilis*. Whereas compounds **5e** and **5g** are moderately effective compared to standard drug streptomycin (MIC = 50 μg mL^{-1}) against gram-negative bacteria *P. aeruginosa and E. coli*. Compound **5e** shows equipotent antifungal activity and **5b** shows moderate antifungal activity compared to standard drug nystatin (MIC = 100 μg mL^{-1}) against fungi *A. parasiticus* and *R. oryzae.*

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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Conflict of Interest

The authors declare no conflict of interest.

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