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Research Article

SYNTHESIS AND STUDY OF 3-METHYL-*N*-[ARYLYLMETHYLENE]-1*H*-INDAZOL-5-AMINES FROM 2-HYDROXY-4-AMINO ACETOPHENONE AND THEIR ANTI-MICROBIAL ACTIVITY

Sujatha K *[1], Jyoti N Rao [3], A.M.A. Khader [2], Balakrishna Kalluraya [2]
[1]Department of Chemistry, Karnataka University, Dharwad, Karnataka, India
[2]Department of Chemistry, Mangalore University, Mangalagangothri, Karnataka, India
[3]Department of P G Studies and Research in Chemistry, St. Alosyus college, Mangalore, Karnataka, India
*Corresponding Author Email: sujathakorganic@gmail.com

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ABSTRACT

Indazole is one of the most important scaffolds in heterocyclic chemistry and drug design and discovery. It is rarely found in nature but widely found in diverse pharmacologically active substances. Indazoles are versatile building-block for lead generation, the indazole core is an active pharmacophore and used in medicinal chemistry and drug discovery research. A number of drugs with indazole core are in the market, In the recent years, many indazole derivatives have been synthesized and subjected to varied biological activities. Here indazoles are prepared from 2-hydroxy-4-amino acetophenone, the free amino group is preprotected and reacted with hydrazine hydrate at elevated temperature thus obtained indazoles are deprotected and hydrazone derivatives were prepared, the newly synthesized indazole derivatives were screened for antibacterial and antifungal activity.

Key words: Indazole, antifungal, hydrazones.

INTRODUCTION

Indazoles is a bicyclic heterocyclic aromatic compound consists of the benzene and pyrazole. Indazoles are very rare in nature, Among the alkaloids nigellicine, nigeglanine, and nigellidine are indazoles. Alkaloid Nigellicine was first isolated from the widely distributed plant *Nigella sativa* L. (black cumin) used in traditional medicine used for treating allergy, Indazole derivatives display a broad spectrum of biological activities[1,2].

The utility of 2H- indazoles as pharmacophore in drug discovery has been exemplified in several recent publications[3-6], a substantial research work around the globe enabling the synthesis of such motifs[7-10]. The planar indazole containing two nitrogen atoms in ring can be functionalized with high selectivity by varying length of side chain and functionalizing at different positions will yield enormous number of indazole derivatives, providing new spectrum of molecules having with therapeutic properties.

The currently available drugs in market such as Granisetron 5HT3 receptor antagonist[11,12], and Benzydamine an anti-inflammatory agent mainly constitute indaole as the core moiety.

Earlier findings on indazole derivatives are specifically known to be active as protein kinase inhibitors viral infections, auto immune and selective 5-HT6R antagonists[13,14] in Alzheimer's neuro degenerative disorders. In recent years, some of the indazole ring systems are being evaluated as potential drugs for variety of physiological activities many compounds approved for clinical use[15].

Thus, many chemists from all over the world are developing different methods for the synthesis of these heterocycles. During the last decade, a considerable interest has been paid to the chemistry of indazoles (benzo[*c*]pyrazole, 1,2- benzodiazole). This is undoubtedly due to a broad variety of biological activities of indazole derivatives which inspired the developments of new syntheses and their optimizations, as well as of functionalization of the indazole ring system.

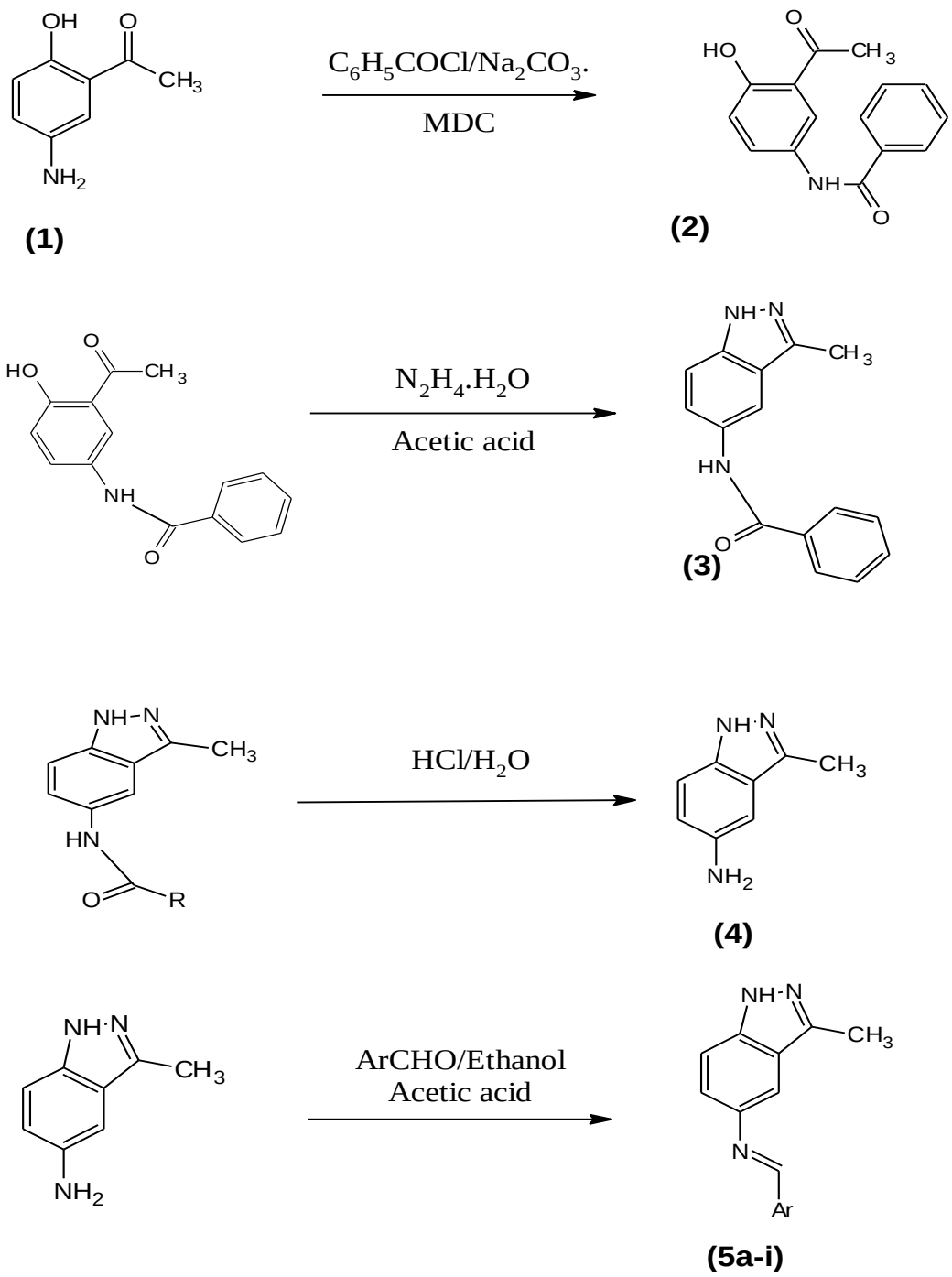
Emil Fisher in 1800 obtained 1,2-dihydroindazol-3-one by heating Ortho-hydrazine benzoic acid named as indazolone and it is also considered as the anhydride of Ortho-hydrazine benzoic acid, Emil Fisher along with Kuzel attempted to obtain the anhydride of ortho-hydrazino cinnamic acid. Among the mixture of products obtained, they were able to isolate a molecule that did not contain any oxygen. Accordingly named as indazole.

Varied methods exist for their synthesis. Two strategies via palladium catalysts are the synthesis of 1-aryl-1H-indazoles via the palladium-catalyzed cyclization of N-aryl-N0-(o-bromobenzyl) hydrazines and [N-aryl-N0-(o-bromo benzyl)-hydrazinato-N0]-triphenylphosphponium bromides and palladium-catalyzed cyclization of arylhydrazones of 2-bromoaldehydes and 2-bromoacetophenones to give 1-aryl-1H-indazoles.

Later the Pd in the preparation of 1-aryl-1H-indazoles was replaced by copper(I)-catalyzed intramolecular amination reaction using CuI, KOH, was developed The Cadogan indazole synthesis, or reductive cyclization of ortho-imino-nitrobenzenes mediated by triethyl phosphite, was one of the first methods described and remains one of the more effective synthetic transformations reported for this purpose.

MATERIALS AND METHODS

The starting material, 2-hydroxy-4-amino acetophenone **(1)** was obtained commercially. Since there is a chance of reaction of amino group, the amino group of 2-hydroxy-4-aminoacetphenone was protected by treating with benzoyl chloride in presence of sodium carbonate as mild base and dichloromethane as solvent. This N-protected hydroxy ketone **(2)** when treated with hydrazine hydrate in acetic acid medium gave *N*-(3-acetyl-4-hydroxyphenyl) benzamide **(3).** Deprotection of the amino group by treating with hydrochloric acid resulted in the formation of 3-methyl-1*H*-indazol-5-amine **(4).** Condensation of indazole amine **(4)** with suitably substituted aromatic aldehydes gave 3-methyl-*N*-[arylylmethylene]-1*H*-indazol-5-amines **(5) (scheme-1)** The newly synthesized compounds were confirmed by analytical data.



Scheme-1

Melting points of the newly synthesized compounds were checked in open capillary tubes and are uncorrected. IR spectra are recorded by dispersing the compounds in KBr pellets on a Schimadzu FT-IR 157 spectrophotometer. ^{1}H NMR spectra were recorded on a 400 MHz Bruker Avance II 400 NMR spectrometer and all the chemical shift values were reported as δ (ppm), downfield from TMS and proton signals are indicated as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Mass spectra were recorded on LCMS (API 3000, Applied Bio Systems) operating at 70eV. Elemental analysis was carried out on an Elementar Vario EL III model. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates.

BIOLOGICAL ACTIVITY

Antimicrobial studies

The newly synthesized compounds were screened for their antibacterial activity *invitro* against Gram-positive bacteria namely *Escherichia coli, Staphylococcus aureus*, and Gram-negative bacteria namely *Pseudomonas aeruginosa, Bacillus subtilis* and the fungus, namely, *Candida albicans* by disc diffusion method. The test compounds were dissolved in N.N-dimethyl formamide (DMF) to obtain a solution of 10µg/ml concentration. The inhibition zones of microbial growth produced by different compounds were measured at the end of an incubation period of 48 hours at 37ºC. DMF alone showed no inhibition zone. Penicillin and Fluconazole were used as reference standards to evaluate the potency of the tested compounds.

General Procedure for Preparation of *N*-(3-acetyl-4-hydroxyphenyl) benzamide (2)

The 1-(5-amino-2-hydroxyphenyl) ethanone was dissolved in water and sodium bicarbonate (2eq) was added with stirring. The resulting solution was cooled to 5˚C and benzoyl chloride (1.5mole) dissolved in MDC was added in drops. The resulting mixture was stirred at 0˚C for 1 hour and allowed to warm to ambient temperature overnight. Water was then added, and the product was extracted into MDC and MDC layer was washed with saturated sodium bicarbonate solution. The combined organic layer was dried over anhydrous sodium sulphate and concentrated under vacuum**.**

General Procedure for Preparation of *N*-(3-methyl-1*H*-indazol-5-yl) benzamide (3)

A mixture of compound **(2)** (0.01 mol) and a molar equivalent of hydrazine hydrate (80%) in glacial acetic acid (15 mL) were heated on water bath at 90 - 95 ºC for 5 - 6 hours. The reaction mass was then poured into ice-cold water. The solid obtained was filtered, washed with water, dried and crystallized from methanol to yield the title compound **(3).**

General Procedure for Preparation of 3-methyl-1*H*-indazol-5- amine (4)

A mixture of protected indazole (10g), 50ml water and 25ml concentrated hydrochloric acid was heated on a water bath for two hours. The reaction mixture was cooled and quenched into ice water mixture with stirring. The precipitated product was filtered and washed with water. The indazole prepared by this procedure is 3-methyl-1*H*-indazol-5- amine, m.p. 120-122^{0}C.

General Procedure for preparation of 3-methyl-*N*-[(substituted phenyl)methylene]-1*H*-indazol-5-amine (5a –K)

To a solution of substituted aldehydes (10 mmol) in methanol (30 mL) was added an equimolar amount of the amino indazole (**4**) and a few drops of concentrated sulfuric acid as a catalyst at room temperature. The reaction mixture was stirred for 8 hours at room temperature. The precipitated compounds were filtered and recrystallized from hot methanol.

RESULTS AND DISCUSSION

The newly synthesized 3-methyl-*N*-[arylmethylene]-1*H*-indazol-5-amines **(5a-k)** were confirmed by evaluating the IR, ^{1}H NMR and mass spectral data. The newly synthesized compounds were screened for their antibacterial and antifungal activities. The characterization data of the newly synthesized compounds were given in **Table 1**.

Table 1: Characterization data of 3-methyl-*N*-[arylmethylene]-1*H*-indazol-5-amines (5a –K)

Compound No	R	Molecular formula	Yield (%)	M.p (^{0}C)
5a	Biphenyl	$C_{21}H_{17}N_3$	80	210-216
5b	Phenyl	$C_{15}H_{13}N_3$	82	137-138
5c	Pyridinyl	$C_{14}H_{12}N_4$	83	124-5
5e	4-Methoxyphenyl	$C_{16}H_{15}N_3O$	85	120
5f	3-Methoxy4-hydroxyphenyl	$C_{16}H_{15}N_3O_2$	82	328-30
5g	3,4-Dimethoxyphenyl	$C_{17}H_{17}N_3O_2$	81	180-82
5h	5-Nitro-3,4-dimethoxyphenyl	$C_{17}H_{16}N_4O_4$	76	179-180
5i	4-Hdroxyphenyl	$C_{15}H_{13}N_3O$	65	210-212
5j	Diethylaminophenyl	$C_{19}H_{22}N_4$	76	136-38
5k	4-Methylphenyl	$C_{16}H_{15}N_3$	68	125-26

Solvent for recrystallisation: ethanol.

The ^{1}H-NMR spectra of *N*-(3-methyl-1*H*-indazol-5-yl) benzamide **(3)** is shown. In this spectrum a singlet at δ, 2.501 is due to the methyl protons integrating for three protons. The multiplets in the region of 6.984-8.123 integrating for eight

protons signal due to aromatic protons of indazole and phenyl ring merged together. The indazole NH proton appeared as a singlet at δ, 12.656 while the amide NH came into resonance as a singlet at δ, 10.231 each integrating for one proton.

In the I.R spectrum of 3-methyl-1 H-indazol-5-amine the NH_2 stretching band was observed in the region of 3300-3400 cm^{-1} and the band at 2961-2924 cm^{-1} may be assigned to C-H stretching.

Further the^{1}H-NMR spectra of 3-methyl-1 H-indazol-5-amine **(4).** The singlet at δ, 2.052 integrating for three protonsl due to

methyl protons appeared. The indazole 4-H appeared as a singlet at δ, 6.68 integrating for one proton. The signal due to other two indazole protons merged together and appeared as multiplets in the region of δ, 6.43-6.51 integrating for two protons. The lone NH proton appeared as singlet at δ, 12.30 integrating for one proton. While the signal due to NH_2 protons appeared as broad singlet at δ, 4.47.

In the IR spectra of (E)-N-(4-(diethylamino) benzylidene)-3-methyl-1H-indazol-5-amine, the absorption bands corresponding to the N-H stretching frequency was observed at 3076 cm^{-1}. Similarly, the characteristic C-H stretching frequency was observed at 2961 cm^{-1} and C=N stretching frequency at 1590 cm^{-1}.

In 3-methyl-*N*-[(1*E*) -(4-methylphenyl) methylene]-1*H*-indazol-5-amine, the two singlets at δ, 2.381and δ, 2.595 integrating for three protons each are due to two methyl groups. Signals due to aromatic protons of indazole merged with aromatic protons of 4-methylphenyl and appeared as multiplets at δ, 6.630-7.744 integrating for seven protons. The N=CH proton appeared as a singlet at δ, 8.619 integrating for one proton, whereas the indazole NH appeared as a singlet at δ, 12.41 integrating for one proton.

Similarly, the ^{1}H-NMR spectrum of *N*-[biphenyl-4-ylmethylene]-3-methyl-1*H*-indazol-5-amine**.** The signals observed are assigned as follows. ^{1}H-NMR (DMSO-d_6): δ, 2.43 (s, 1H, CH_3), 7.158-7.986 (m, 13H, Ar-H), 8.80 (s, 1H, N=CH), 12.10 (s, 1H, NH).

Further evidence for the formation of the compounds was obtained from mass spectra. Mass spectrum of compound (E)-N-(4-(methyl) benzylidene)-3-methyl-1H-indazol-5-amine, the molecular ion peak M+1 was observed at m/z, 250 consistent with the molecular formula $C_{16}H_{15}N_3$. Similarly, for the compound (E)-N-(4-(diethylamino) benzylidene)-3-methyl-1H-indazol-5-amine the molecular ion M+1 peak was observed at m/z, 307 is consistent with molecular formula $C_{19}H_{22}N_4$.

The results are for Antimicrobial are presented in the Table 2.

Table 2: Antibacterial and antifungal data of compound (5a-l)

Compound No	Antibacterial activity (MIC in µg/mL)				Antifungal activity (MIC in µg/mL)
	E. coli	***S. aureus***	***P. aeruginosa***	***B. subtilis***	***C. albicans***
5a	12.5	25	6.25	12.5	6.25
5b	6.25	6.25	6.25	6.25	12.5
5c	6.25	3.125	6.25	12.5	3.125
5d	6.25	12.5	6.25	6.25	-
5e	12.5	12.5	6.25	12.5	6.25
5f	6.25	6.25	3.125	6.25	12.5
5g	3.125	6.25	3.125	3.125	6.25
5h	6.25	6.25	6.25	6.25	6.25
5l	6.25	12.5	3.125	12.5	6,25
5j	-	-	-	-	-
5k	6.25	6.25	6.25	12.5	6.25
Standard: Penicillin	0.12	0.12	0.12	0.12	---
Standard: Fluconazole	-	-	-	-	8.0
Control: DMF	-	-	----	---	---

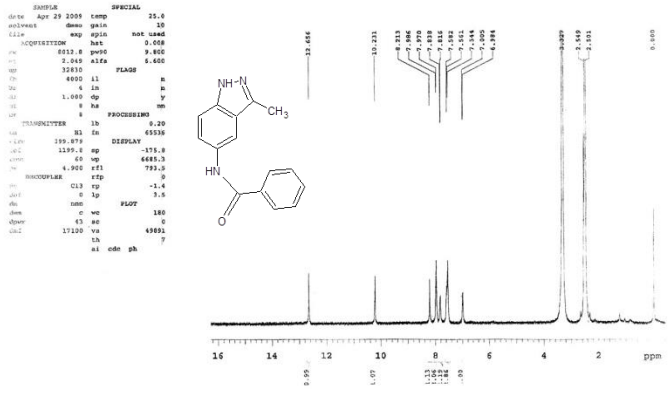


Figure 1: ^{1}H NMR Spectrum of compound *N*-(3-methyl-1*H*-indazol-5-yl) benzamide (3)

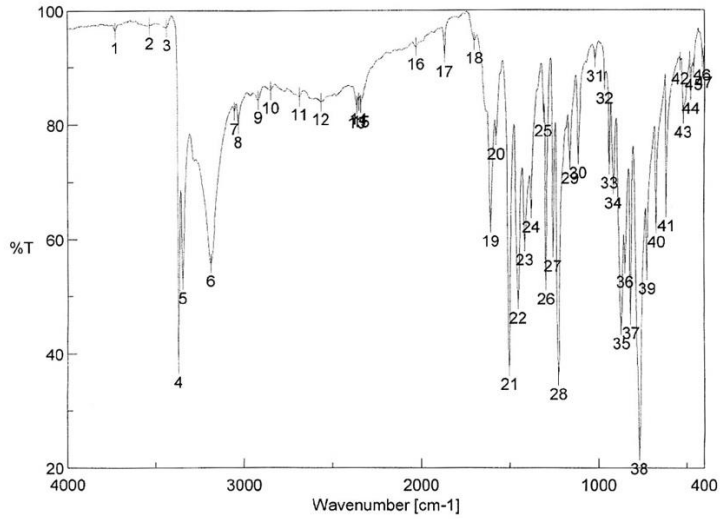


Figure 2: IR Spectrum of compound 3-methyl-1*H*-indazol-5- amine (4)

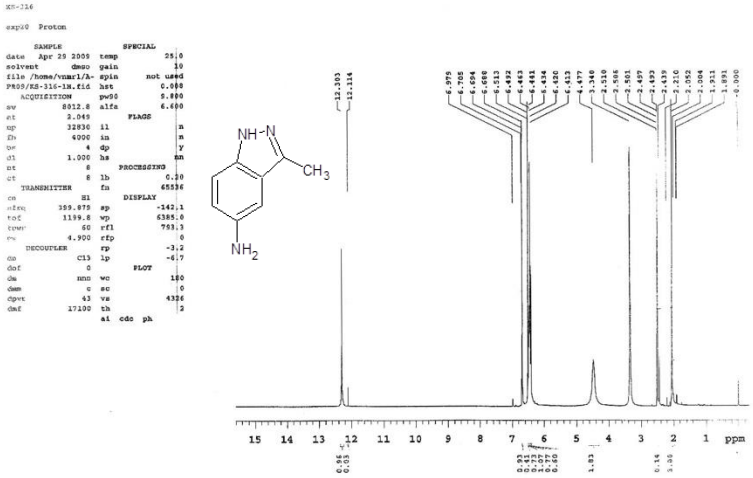


Figure 3: ^{1}H NMR Spectrum of compound 3-methyl-1*H*-indazol-5- amine (4)

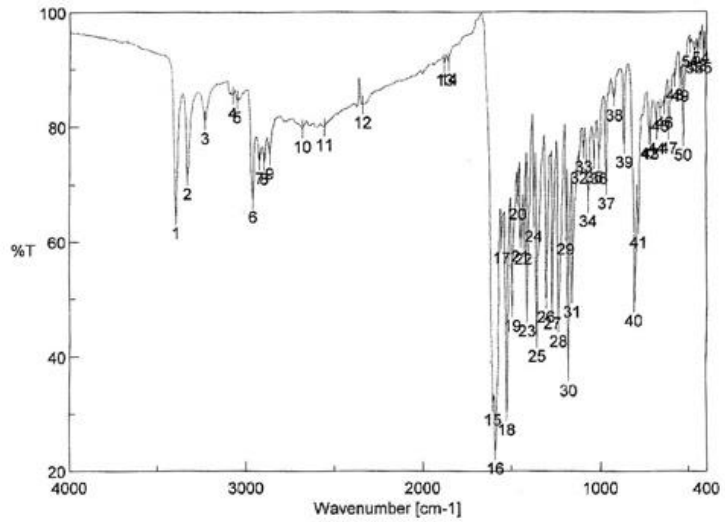


Figure 4: IR Spectrum of compound (E)-N-(4-(diethylamino) benzylidene)-3-methyl-1H-indazol-5-amine 5j

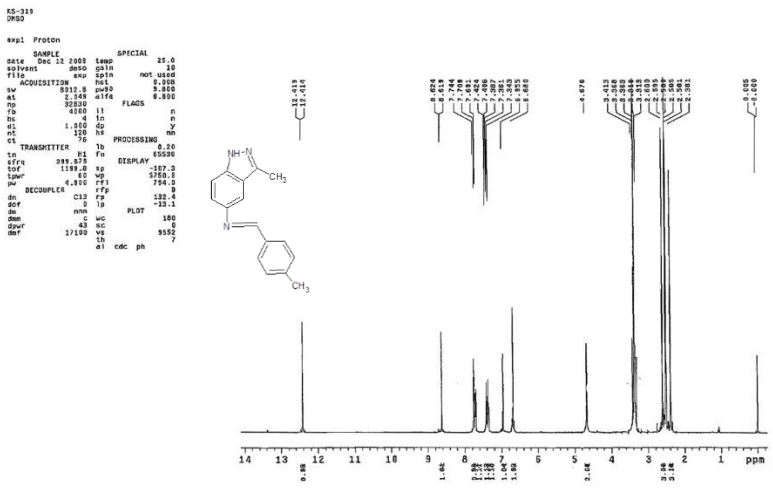


Figure 5: ^{1}H NMR Spectrum of compound (E)-N-(4-(methyl) benzylidene)-3-methyl-1H-indazol-5-amine 5K

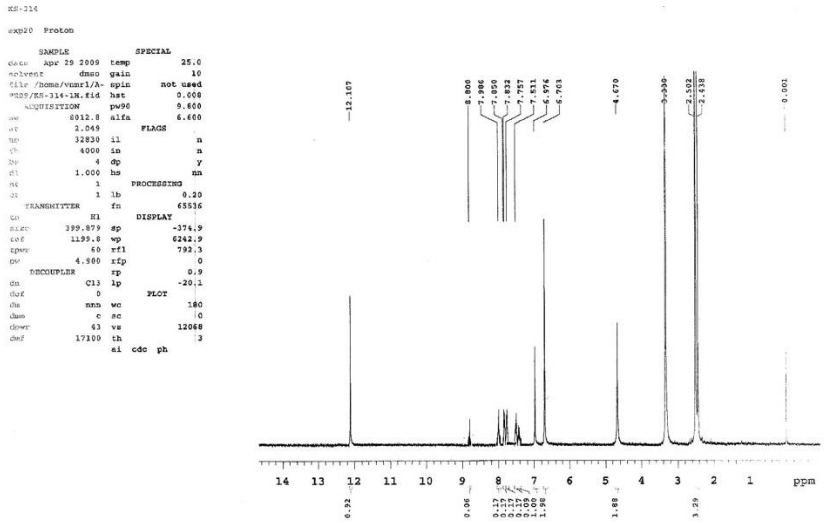


Figure 6: ^{1}H NMR Spectrum of compound *N*-[biphenyl-4-ylmethylene]-3-methyl-1*H*-indazol-5-amine. (5a)

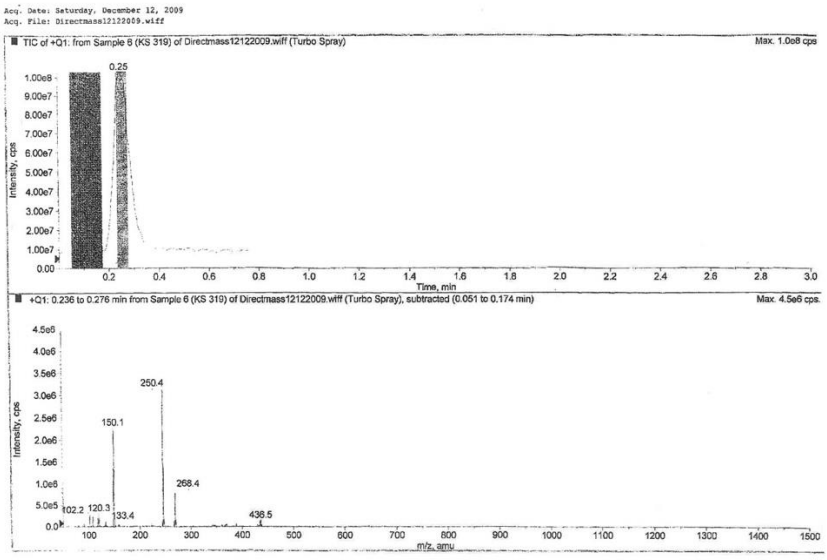


Figure 7: Mass Spectrum of compound (E)-N-(4-(methyl) benzylidene)-3-methyl-1H-indazol-5-amine 5K

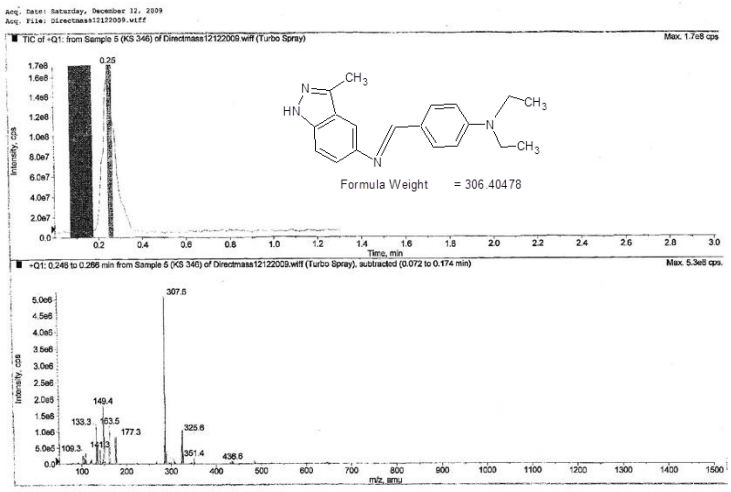


Figure 8: Mass Spectrum of compound (E)-N-(4-(diethylamino) benzylidene)-3-methyl-1H-indazol-5-amine 5j

This study reports the successful synthesis of the title compounds. The antimicrobial activity study revealed that some of the substituted indazole possess antifungal activity.

CONCLUSION

This work describe the synthesis of Indazole derivatives, The synthesized compounds were characterized by IR, ^{1}HNMR and mass spectral studies, all compounds were screened for antibacterial and antifungal activity, Out of the sixteen compounds hereby reported that compound **5c** showed the best activity against microbial strains *Candida albicans* with an MIC of 3.12 mg/ml. The activity is found to be better than standard.

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