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Original article

$GdCl_3$ catalysed Grieco condensation: A facile approach for the synthesis of novel pyrimidine and annulated pyrimidine fused indazole derivatives in single pot under mild conditions and their anti-microbial activity ☆

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Abstract

Indazole regioisomers such as 3-amino-4-(trifluoromethyl)-6-phenyl-1*H*-indazole-7-carbonitrile **1** and 3-amino-6-(trifluoromethyl)-4-phenyl-1*H*-indazole-7-carbonitrile **2** were independently reacted with formaldehyde followed by unsymmetrical, symmetrical and cyclic electron rich olefins in presence of $GdCl_3$ as catalyst and obtained pyrimidine fused indazole derivatives **3** and **4**, respectively. The reaction is found to be concerted and an exclusive product is formed. Representative examples of compounds **3** and **4** were screened against Gram-positive, Gram-negative bacteria and fungal species such as yeast and filamentous fungi in vitro. Compound **3f** showed significant activity against all species of Gram-positive and Gram-negative bacteria, whereas compounds **3h** and **4a** showed the least activity with reference to penicillin as well as streptomycin. Similarly compound **3c** showed promising activity against yeast and filamentous fungi whereas compound **3f** is inactive at the maximum concentration of 150 μg/mL.

Keywords: Regioisomers; Indazoles; Pyrimidines; Cyclisation; Olefins; Diazadiene

1. Introduction

The modern trend in organic synthesis is driving towards discovery of new organic molecules as potential drugs by adopting various synthetic strategies. Among the prominent ring systems such as pyrazole-fused benzenes are also called indazole derivatives, known to be active against cancer cell proliferative disorders [1], protein kinases [2,3], Alzeimer's diseases [4]. Similarly pyrimidine ring in an organic molecule also shows prominent activity as anti-malarial [5], anti-infective agents [6], and pyridoxol antagonist diseases [7]. Therefore interest is continuously increasing on fusion of pyrimidine ring over indazoles as a result, pyrimidine fused indazoles are formed which are considered to have promising activity against many infections. Thus our attention was directed towards synthesis of a series of novel pyrimidine fused indazole derivatives with trifluoromethyl group at an appropriate position to find an organic molecule as potential drug.

Paul A. Grieco and Ali Bhasas [8] for the first time in 1988 devised a methodology for three-component condensation reaction called Grieco condensation. It involves reaction of amine with aldehydes followed by electron rich olefins to have tetrahydro quinolines. Thus multicomponent reactions overriding linear reactions to combat the environmental legislations. Earlier reports based on Grieco condensation were mainly about aromatic amines [9–12] condensed with formaldehyde to have azadiene followed by reaction with electron rich olefins in order to get tetrahydroquinolines. Alternately imines [13,14] are generated from amines and carbonyl compounds

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then reacted with various dienes to have cyclo addition products. However, generation of diazadiene is not known so far except our report [15]. In continuation of our efforts [16–19], we have chosen 3-amino indazole regioisomers **1** and **2** [20] and reacted with formaldehyde as a result, diazadienes are formed in situ followed by cyclisation with various electron rich olefins resulted in novel pyrimidine fused indazole derivatives **3** and **4**, respectively. Representative examples were screened for anti-bacterial and anti-fungal activity. Compounds **3f** and **3c** are considered as interesting lead compounds and further studies are in progress to develop more potent anti-bacterial and anti-fungal compounds, respectively.

2. Chemistry

The regioisomers of indazoles such as 3-amino-4-(trifluoromethyl)-6-phenyl-1*H*-indazole-7-carbonitrile **1** and 3-amino-6-(trifluoromethyl)-4-phenyl-1*H*-indazole-7-carbonitrile **2** were selected as suitable amines for generation of diazadienes, as amine functional group is in conjugation with imine in pyrazole ring. Thus compounds **1** and **2** were independently reacted with formaldehyde in acetonitrile at room temperature and diazadienes were formed in situ. The generated diazadienes are reacted with various substituted electron rich olefins using $GdCl_3$ as catalyst. Solvent was removed and residue is treated with water, extraction with dichloromethane and column chromatography resulted in cyclic products **3** and **4**, respectively, in high yields. A typical sequence of reaction is drawn in Scheme 1.

The mode of reaction is mainly formation of Schiff's base in situ, which acts as a diazadiene and is complexed with $GdCl_3$. The complex is further reacted with various electron rich alkenes in order to get cyclised adducts. In a typical example when isoprene, an unsymmetrical olefin is reacted with diazadiene, an exclusive product **3h** or **4h** is formed depending upon the regioisomer. It is attributed to an attack of nucleophile from diazadiene on tertiary carbon of olefin as a result, only one product is formed, as unsaturated tertiary carbon being relatively more nucleophilic than unsaturated secondary carbon followed by primary carbon. The probable sequence of mechanism is drawn in Scheme 2.

In order to see the versatility of the reaction, indazole regioisomers **1** and **2** were further reacted with formaldehyde followed by various cyclic olefins and cis products were obtained exclusively in high yields. The structure and stereochemistry of all the products were identified based on IR, ^{1}H NMR, mass spectra and elemental analysis data. The small coupling constant (J) of the ring junction protons (4a*S*,12a*S*) of 9.6 Hz in compound **3a** indicates a cis ring junction and is in agreement with earlier reports [11,12]. The similar trend is followed in other compounds and products formed in each case are tabulated in Table 1.

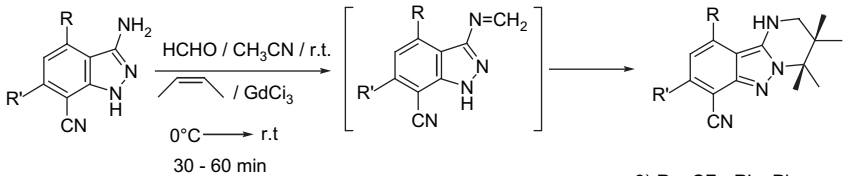
3. In vitro anti-bacterial assays

Four bacterial test organisms such as *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 1688), and *Escherichia coli* (MTCC 443) were selected and obtained from the Institute of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on Nutrient agar slants and were sub-cultured in Petri dishes prior to testing. The media used was Nutrient agar, Nutrient broth procured from Himedia Laboratories, Mumbai. The minimum inhibitory concentration was determined by broth dilution method [21].

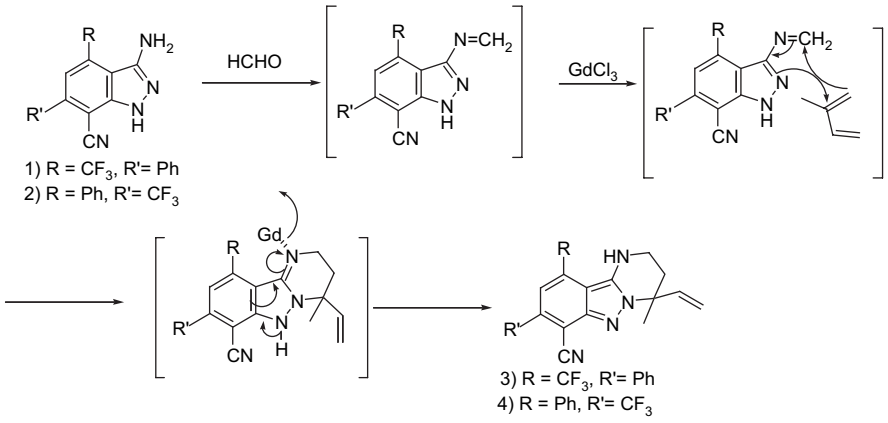
Compounds **3a–h**, **4a** and **4h** were dissolved in acetone and screened for their in vitro anti-bacterial activity. The anti-bacterial activity was tested against Gram-positive (*B. subtilis*, *S. aureus)* and Gram-negative (*P. aeruginosa*, *E. coli*) bacteria in vitro. Compound **3f** showed significant activity against all species of Gram-positive and Gram-negative bacteria with reference to penicillin as well as streptomycin. Compounds **3d**, **3e** and **3g** showed moderate activity whereas **3h** and **4a** showed the least activity. Among regioisomers **3a** and **4a**, compound **3a** is more active than compound **4a** against *S. aureus*. Similarly among other regioisomers **3h** and **4h**, compound **4h** is more active than compound **3h** against *B. subtilis* and *P. aeruginosa* and less active against *S. aureus*. In conclusion 4,8-diphenyl-10-(trifluoromethyl)-1,2,3,4-tetrahydropyrimido[1,2-*b*]indazole-7-carbonitrile **3f** is the most active among the screened compounds while compounds **3h** and **4a** showed the least activity. The MIC values of the compounds were compared with those obtained with penicillin and streptomycin. The details of compounds and their activity against various microorganisms are tabulated in Table 2.

4. In vitro anti-fungal activity assays

The in vitro anti-fungal activity of compounds **3a–h**, **4a** and **4h** was screened against the fungal strains, viz., *Candida*



Scheme 1.

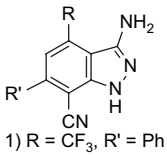


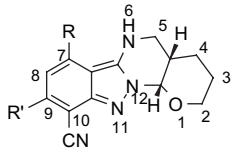
Scheme 2.

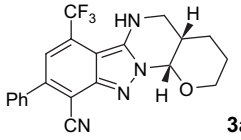
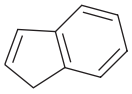
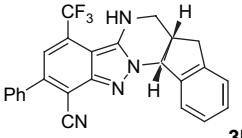
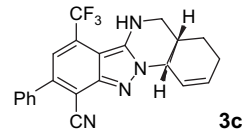
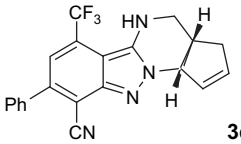
albicans (MTCC 227), *Saccharomyces cereviseae* (MTCC 36) and filamentous fungal cultures like *Rhizopus oryzae* (MTCC 262) and *Aspergillus niger* (MTCC 1344) by agar cup diffusion method [22]. The strains were obtained from the Institute of Microbial Technology, Chandigarh.

Compound **3c** showed promising activity against all the fungi at different concentrations except with *S. cereviseae*. It is found that the inhibition diameter is concentration dependent and increases with concentration. Compound **3f** is inactive against all the fungal cultures screened. Comparing the

Table 1
Preparation of annulated pyrimidine indazole derivatives **3a–h**, and **4a–h**

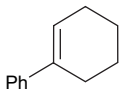
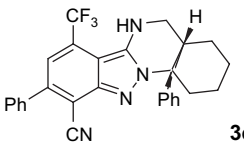
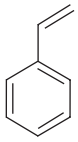
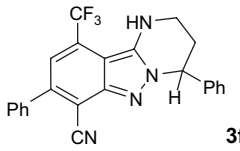
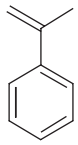
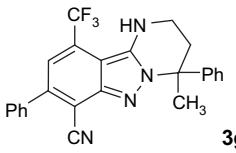
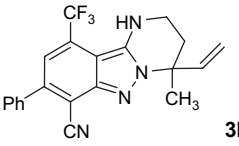




Alkene	Product	Yield (%)	Product	Yield (%)
	 **3a**	85	**4a**	84
	 **3b**	88	**4b**	89
	 **3c**	89	**4c**	91
	 **3d**	94	**4d**	96

(*continued on next page*)

Table 1 (*continued*)

Alkene	Product	Yield (%)	Product	Yield (%)
	 **3e**	89	**4e**	87
	 **3f**	93	**4f**	95
	 **3g**	91	**4g**	93
	 **3h**	78	**4h**	79

regioisomers **3a** and **4a**, compound **4a** is found to be more active. Similarly regioisomer **4f** is more active than **3f**. Compounds **3e** and **3g** also showed significant activity. However, all compounds **3a**–**h**, **4a** and **4f** are inactive against yeast fungal culture *S. cereviseae* upto a maximum concentration of 150 μg/mL. In conclusion, annulated pyrimidine fused indazoles are more active than pyimidine fused indazoles. The inhibitory zone diameters of the compounds are compared with those obtained with 50 μg/mL. The details of the results have been tabulated in Table 3.

Table 2
Minimum inhibitory concentration (MIC in μg/mL) of compounds **3a**–**h**, **4a** and **4h** for various bacterial microorganisms

Compound	Microorganism			
	Gram-positive		Gram-negative	
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*
3a	150.0	75.0	150.0	75.0
3b	75.0	150.0	150.0	75.0
3c	150.0	75.0	75.0	37.5
3d	150.0	37.5	75.0	37.5
3e	75.0	37.5	75.0	37.5
3f	50.0	50.0	50.0	25.0
3g	75.0	75.0	75.0	37.5
3h	150.0	75.0	150.0	150.0
4a	150.0	150.0	150.0	75.0
4h	75.0	150.0	75.0	150.0
Penicillin	1.562	1.562	6.25	12. 5
Streptomycin	6.25	6.25	1.562	1.562

Negative control; acetone showed no activity.

5. Conclusion

A single pot method has been developed for the synthesis of pyrimidine fused and annulated pyrimidine fused indazoles using $GdCl_3$ as catalyst in high yields. Representative examples were screened against Gram-positive, Gram-negative bacteria, yeasts and filamentous fungi. Compound **3f** showed promising activity against bacteria and compound **3c** against fungi.

6. Experimental

Melting points were recorded on Casia-Siamia (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectrophotometer using KBr optics. ^{1}H NMR spectra were recorded on Varian Gemini varion 200 MHz, Bruker AV 300 MHz and Unity 400 MHz spectrometer in $CDCl_3$ using TMS as an internal standard. LSIMS mass spectra were recorded on a VG 7070 H instrument at 70 eV. All reactions were monitored by thin layer chromatography (TLC) on precoated silicagel 60 F_{254} (mesh); spots were visualized with UV light. Merck silicagel

Table 3
Inhibitory zone diameters of compounds **3a–h**, **4a** and **4h** for various fungal cultures at different concentrations

Compound	*C. albicans* (μg/mL)		*S. cerviseae* (μg/mL)		*R. oryzae* (μg/mL)		*A. niger* (μg/mL)	
	100	150	100	150	100	150	100	150
3a	–	–	–	–	9 mm	–	–	–
3b	–	–	–	–	7 mm	9 mm	–	9 mm
3c	7 mm	8 mm	–	–	8 mm	12 mm	10 mm	12 mm
3d	–	9 mm	–	–	–	–	–	10 mm
3e	9 mm	11 mm	–	–	–	9 mm	–	10 mm
3f	–	–	–	–	–	–	–	–
3g	8 mm	9 mm	–	–	–	12 mm	–	–
3h	7 mm	8 mm	–	–	–	–	7 mm	8 mm
4a	8 mm	10 mm	–	–	–	9 mm	–	–
4f	–	–	–	–	8 mm	10 mm	–	–
Amphotericin-B (50 μg/mL)	23.5 mm		22 mm		23 mm		26 mm	

Negative control; DMSO showed no activity; well or cup method; the concentrations are expressed in μg/mL and inhibitory zone diameters are expressed in mm.

(60–120 mesh) was used for chromatography. CHN analyses were recorded on a Vario EL analyzer.

6.1. General procedure

6.1.1. Preparation of annulated pyrimidine fused indazoles (*3a–h* and *4a–h*)

To a solution of indazole regioisomers (**1** or **2**) (500 mg, 1.65 mmol) in acetonitrile (8 mL), 40% formaldehyde solution (3 mL) was added and the mixture was stirred for 15–20 min under N_2 atmosphere at room temperature. The solution was cooled to 0 °C and $GdCl_3$ (2.64 mg, 10 mol%) was added followed by the addition of corresponding olefin (2 equiv.). The total mixture was stirred for 30–60 min, under N_2 atmosphere at room temperature. Acetonitrile was removed under vacuo and the residue reaction mixture was treated with water, extracted with dichloromethane and dried over sodium sulphate. The dichloromethane extract was concentrated to give the crude products **3** or **4** and were purified by column chromatography using 60–120 mesh silicagel. The desired products were eluted with 20% EtOAc/hexane mixture and obtained in high yields.

*6.1.1.1. (4aS,12aS)-9-Phenyl-7-(trifluoromethyl)-3,4,4a,5,6,12a-hexahydro-2H-pyrano[3′,2′,5,6]pyrimido[1,2-b]indazole-10-carbonitrile (**3a**).* Yield: 560 mg (85%); yellow coloured solid; mp +300 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.41$–1.62 (1H, m, OCH_2CH_2), 2.12–2.18 (1H + 2H, m, OCH_2CH_2, CH_2–CH), 2.24–2.42 (1H, m, C*H*), 3.26–3.48 (1H, m, CH_2–N), 3.64–3.96 (1H + 1H, m, CH_2–O, CH_2–N), 4.12–4.16 (1H, m, CH_2–O), 5.68–5.76 (1H, d, *J* 9.6 Hz, *H*C–O), 5.84 (1H, br s, N*H*), 7.14 (1H, s, H–C(8), Ar-H), 7.42–7.85 (5H, m, Ar-H). IR (KBr): 3381 (N–H), 2934 (C–H), 2229 (C≡N), 1606 (C=N), 2210 (CN) cm^{-1}. LSIMS *m/z*: 399 (MH^+). Anal. calcd. for $C_{21}H_{17}F_3N_4O$: C, 63.31; H, 4.30; N, 14.06; found: C, 63.10; H, 4.11; N, 14.24%.

*6.1.1.2. (6aS,11aS)-2-Phenyl-4-(trifluoromethyl)-6,6a,11,11a-tetrahydro-5H-indeno[1′,2′:5,6]pyrimido[1,2-b]indazole-1-carbonitrile (**3b**).* Yield: 620 mg (88%); bright yellow coloured solid; mp 203 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.82$–2.90 (2H + 1H + 1H, m, CH_2, *CH*, CH_2–N), 3.58–3.68 (1H, m, CH_2–N), 5.52 (1H, br s, NH), 5.7 (1H, d, *J* 8.9 Hz, *H*C–N), 7.8 (1H, m, Ar-H), 7.20–7.30 (3H, m, Ar-H), 7.48–7.68 (5H, m, Ar-H), 7.85–7.98 (1H, m, Ar-H). IR (KBr): 3356 (N–H), 2933 (C–H), 2226 (C≡N), 1606 (C=N) cm^{-1}. LSIMS *m/z*: 431 (MH^+). Anal. calcd. for $C_{25}H_{17}F_3N_4$: C, 69.76; H, 3.98; N, 13.01; found: C, 69.97; H, 4.48; N, 12.77%.

*6.1.1.3. (4aS,12aS)-9-Phenyl-7-(trifluoromethyl)-3,4,4a,5,6,12a-hexahydroindazolo[2,3-a]quinazoline-10-carbonitrile (**3c**).* Yield: 580 mg (89%); yellow coloured solid; mp 175 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.83$–2.0 (2H, m, CH_2–CH_2–HC=CH), 2.06–2.24 (2H, m, CH_2–HC=CH, C*H*), 2.48–2.56 (1H, m, *CH*), 3.42–3.58 (2H, m, CH_2–N), 5.14 (1H, s, HC–N), 5.60 (1H, br s, N*H*), 5.92 (1H, d, *J* 17.9 Hz, *H*C=CH), 6.20 (1H, m, HC=C*H*), 7.40–7.56 (4H, m, Ar-H), 7.58–7.68 (2H, m, Ar-H). IR (KBr): 3405 (N–H), 2936 (C–H), 2228 (C≡N), 1615 (C=N) cm^{-1}. LSIMS *m/z*: 395 (MH^+). Anal. calcd. for $C_{22}H_{17}F_3N_4$: C, 66.99; H, 4.34; N, 14.20; found: C, 66.81; H, 4.59; N, 13.90%.

*6.1.1.4. (3aS,11aS)-8-Phenyl-6-(trifluoromethyl)-3a,4,5,11a-tetrahydro-1H-cyclopenta[5,6]pyrimido[1,2-b]indazole-9-carbonitrile (**3d**).* Yield: 590 mg (94%); yellow coloured solid; mp 186 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.2$ (2H, m, CH_2), 2.3–2.48 (1H, m, C*H*), 2.68–2.88 (1H, m, CH_2–N), 3.12–3.28 (1H, m, CH_2–N), 3.46–3.58 (1H, m, C*H*–N), 5.70 (1H, br s, N*H*), 5.96 (1H, d, *J* 17.1 Hz, *H*C=CH), 6.20 (1H, d, *J* 17.1 Hz, HC=C*H*), 7.40–7.65 (6H, m, Ar-H). IR (KBr): 3351 (N–H), 2928 (C–H), 2230 (C≡N), 1604 (C=N) cm^{-1}. LSIMS *m/z*: 381(MH^+). Anal. calcd. for $C_{21}H_{15}F_3N_4$: C, 66.31; H, 3.97; N, 14.72; found: C, 66.54; H, 3.81; N, 14.89%.

*6.1.1.5. (4aS,12aS)-9,12a-Diphenyl-7-(trifluoromethyl)-1,2,3,4,4a,5,6,12a-octahydroindazolo[2,3-a]quinazoline-10-carbonitrile (**3e**).* Yield: 690 mg (89%); bright yellow coloured solid; mp 266 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.4$–1.6 (2H + 1H, m, CH_2CH_2–CH, CH_2CH_2–CPh), 1.68–1.81 (2H + 1H, m, CH_2–HC, CH_2CH_2–CPh), 1.95–2.05 (1H, m, CH_2–CPh), 2.43 (1H, d, *J* 16.8 Hz, C*H*), 3.0–3.1 (1H, m,

CH_2–CPh), 3.14–3.22 (2H, m, CH_2–N), 5.55 (1H, br s, N*H*), 6.65–6.75 (2H, m, Ar-H), 7.10–7.28 (4H, m, Ar-H), 7.41–7.55 (3H, m, Ar-H), 7.58–7.68 (2H, m, Ar-H). IR (KBr): 3343 (N–H), 2932 (C–H), 2228 (C≡N), 1606 (C=N) cm^{-1}. LSIMS *m/z*: 473 (MH^+). Anal. calcd. for $C_{28}H_{23}F_3N_4$: C, 71.17; H, 4.90; N, 11.85; found: C, 71.35; H, 5.12; N, 11.61%.

*6.1.1.6. 4,8-Diphenyl-10-(trifluoromethyl)-1,2,3,4-tetrahydropyrimido[1,2-b] indazole-7-carbonitrile (**3f**).* Yield: 0.64 g (93%); bright yellow coloured solid, mp +300 °C, ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.48$–2.62 (2H, m, CH_2), 3.26–3.58 (2H, m, CH_2–N), 5.82 (1H, br s, NH), 5.84 (1H, t, *J* 8.2 Hz, C*H*-Ph), 6.92 (2H, d, *J* 16.8 Hz, Ar-H), 7.18–7.3 (1H, s, Ar-H), 7.26–7.41 (3H, m, Ar-H), 7.42–7.58 (3H, m, Ar-H), 7.59–7.72 (2H, d, *J* 16.8 Hz, Ar–H). IR (KBr): 3470 (N–H), 2929 (C–H), 2232 (C≡N), 1609 (C=N) cm^{-1}. LSIMS *m/z*: 419 (MH^+), Anal. calcd. for $C_{24}H_{17}F_3N_4$: C, 68.89; H, 4.09; N, 13.39; found: C, 68.73; H, 3.88; N, 13.59%.

*6.1.1.7. 4-Methyl-4,8-diphenyl-10-(trifluoromethyl)-1,2,3,4-tetrahydropyrimido[1,2-b]indazole-7-carbonitrile (**3g**).* Yield: 650 mg (91%); yellow coloured solid; mp 193 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.15$ (3H, s, CH_3), 2.32–2.48 (1H, m, C–CH_2), 2.52–2.65 (1H, m, C–CH_2), 3.05–3.15 (1H, m, CH_2–N), 3.41–3.58 (1H, m, CH_2–N), 5.85 (1H, br s, N*H*), 6.81–6.92 (2H, m, Ar-H), 7.12 (1H, s, Ar-H), 7.22–7.38 (3H, m, Ar-H), 7.48–7.61 (3H, m, Ar-H), 7.62–7.72 (2H, m, Ar-H). IR (KBr): 3398 (N–H), 2934 (C–H), 2226 (C≡N), 1605 (C=N) cm^{-1}. ESIMS *m/z*: 433 (MH^+). Anal. calcd. for $C_{25}H_{19}F_3N_4$: C, 69.43; H, 4.42; N, 12.95; found: C, 69.68; H, 4.24; N, 12.80%.

*6.1.1.8. 4-Methyl-8-phenyl-10-(trifluoromethyl)-4-vinyl-1,2,3,4-tetrahydropyrimido[1,2-b]indazole-7-carbonitrile (**3h**).* Yield: 490 mg (78%); yellow coloured solid; mp 174 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.84$ (3H, s, CH_3–C), 2.1–2.24 (2H, m, CH_2), 3.48–3.58 (2H, m, CH_2–NH), 4.71 (1H, d, *J* 18.4 Hz, HC=CH_2), 5.22 (1H, d, *J* 18.5 Hz, HC=CH_2), 5.5 (1H, br s, N*H*), 5.95–6.1 (1H, m, *H*C=CH_2), 7.4–7.68 (6H, m, Ar-H). IR (KBr): 3395 (N–H), 2931 (C–H), 2221 (C≡N), 1606 (C=N) cm^{-1}. LSIMS *m/z*: 383 (MH^+). Anal. calcd. for $C_{21}H_{17}F_3N_4$: C, 65.96; H, 4.48; N, 14.65; found: C, 65.80; H, 4.71; N, 14.82%.

*6.1.1.9. (4aS, 12aS)-7-Phenyl-9-(trifluoromethyl)-3,4,4a,5,6,12a-hexahydro-2H-pyrano[3′,2′:5,6] pyrimido [1,2-b] indazole-10-carbonitrile (**4a**).* Yield: 550 mg (84%); yellow coloured solid; mp +300 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.48$–1.53 (2H, m, OCH_2CH_2), 1.85–2.04 (2H, m, OCH_2CH_2–CH_2–CH), 2.56–2.61 (1H, m, C*H*), 3.22–3.51 (1H, m, CH_2–N), 3.74–3.9 (2H, m, CH_2–O, CH_2–N), 4.01–4.18 (1H, m, CH_2–O), 5.19 (1H, d, *J* 9.6 Hz, *H*C–O), 5.78 (1H, br s, N*H*), 7.42–7.61 (6H, m, Ar-H). IR (KBr): 3387 (N–H), 2938 (C–H), 2226 (C≡N), 1607 (C=N) cm^{-1}. LSIMS *m/z*: 399 (MH^+). Anal. calcd. for $C_{21}H_{17}F_3N_4O$: C, 63.31; H, 4.30; N, 14.06; found: C, 63.15; H, 4.53; N, 14.23%.

*6.1.1.10. (6aS,11aS)-4-Phenyl-2-(trifluoromethyl)-6,6a,11,11a-tetrahydro-5H-indeno[1′,2′:5,6]pyrimido[1,2-b]indazole-1-carbonitrile (**4b**).* Yield: 630 mg (89%); bright yellow coloured solid; mp +300 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.91$–3.08 (2H + 1H, m, CH_2, CH_2–N), 3.25–3.40(1H, m, C*H*), 3.48 (1H, m, CH_2–N), 4.85 (1H, br s, NH), 5.95 (1H, d, *J* 8.9 Hz, *H*C–N), 7.21–7.29 (4H, m, Ar-H), 7.48–7.58 (6H, m, Ar-H). IR (KBr): 3359 (N–H), 2928 (C–H), 2228 (C≡N), 1603 (C=N) cm^{-1}. LSIMS *m/z*: 431 (MH^+). Anal. calcd. for $C_{25}H_{17}F_3N_4$: C, 69.76; H, 3.98; N, 13.01; found: C, 70.01; H, 3.80; N, 12.86%.

*6.1.1.11. (4aS,12aS)-7-Phenyl-9-(trifluoromethyl)-3,4,4a,5,6,12a-hexahydroindazolo[2,3-a]quinazoline-10-carbonitrile (**4c**).* Yield: 590 mg (89%); yellow coloured solid; mp +300 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.80$–1.96 (2H, m, CH_2–CH_2–HC=CH), 2.12–2.26 (2H, m, CH_2–HC=CH), 2.51–2.55 (1H, m, C*H*), 3.40–3.56 (2H, m, CH_2–N), 5.18 (1H, s, HC–N), 5.62 (1H, br s, N*H*), 5.90 (1H, d, *J* 18 Hz, *H*C=CH), 6.22 (1H, d, *J* 17 Hz, HC=C*H*) 6.96–7.12 (3H, m, Ar-H), 7.22–7.42 (3H, m, Ar-H). IR (KBr): 3405 (N–H), 2934 (C–H), 2233 (C≡N), 1610 (C=N) cm^{-1}. LSIMS *m/z*: 395(MH^+). Anal. calcd. for $C_{22}H_{17}F_3N_4$: C, 66.99; H, 4.34; N, 14.20; found: C, 66.83; H, 4.13; N, 14.40%.

*6.1.1.12. (3aS,11aS)-6-Phenyl-8-(trifluoromethyl)-3a,4,5,11a-tetrahydro-1H-cyclopenta[5,6]pyrimido[1,2-b]indazole-9-carbonitrile (**4d**).* Yield: 60 mg (96%); yellow coloured solid; mp 218 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.22$ (2H, m, CH_2), 2.32–3.51 (1H, m, CH), 2.70–2.89 (1H, m, CH_2–N), 3.18–3.30 (1H, m, CH_2–N), 5.56 (1H, d, *J* 7.5 Hz, *H*C–N) 5.68 (1H, br s, N*H*), 5.98 (1H, d, *J* 17.8 Hz, *H*C=CH), 6.25 (1H, d, *J* 17.8 Hz, HC=C*H*), 7.58–7.68 (4H, m, Ar-H), 8.08–8.18 (2H, m, Ar-H). IR (KBr): 3352 (N–H), 2933 (C–H), 2229 (C≡N), 1604 (C=N), 2928 (C–H) cm^{-1}. LSIMS *m/z*: 381(MH^+). Anal. calcd. for $C_{21}H_{15}F_3N_4$: C, 66.31; H, 3.97; N, 14.72; found: C, 66.49; H, 4.19; N, 14.48%.

*6.1.1.13. (4aS)-7,12a-Diphenyl-9-trifluoromethyl)1,2,3,4,4a,5,6,12a-octahydroindazolo[2,3-a]quinazoline-10-carbonitrile (**4e**).* Yield: 670 mg (87%); bright yellow coloured solid; mp 242 °C. ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.48$–1.61 (2H + 1H, m, CH_2CH_2–CH, CH_2CH_2–CPh), 1.70–1.90 (2H + 1H, m, CH_2–HC, CH_2CH_2–CPh), 2.02–2.22 (1H, m, CH_2–CPh), 2.48 (1H, d, *J* 16 Hz, C*H*), 3.02–3.10 (1H, m, CH_2–CPh), 3.20–3.36 (2H, m, CH_2–N), 4.90 (1H, br s, N*H*), 6.70–6.79 (2H, m, Ar-H), 6.92 (1H, s, Ar-H), 7.24–7.32 (3H, m, Ar-H), 7.51–7.61(5H, m, Ar-H). IR (KBr): 3322 (N–H), 2932 (C–H), 2230(C≡N), 1605 (C=N), 2932 (C–H) cm^{-1}. LSIMS *m/z*: 472 (MH^+). Anal. calcd. for $C_{28}H_{23}F_3N_4$: C, 71.17; H, 4.90; N, 11.85; found: C, 71.40; H, 4.74; N, 12.02%.

*6.1.1.14. 4,10-Diphenyl-8-(trifluoromethyl)-1,2,3,4-tetrahydropyrimido[1,2-b]indazole-7-carbonitrile (**4f**).* Yield: 650 mg (95%); bright yellow coloured solid; mp 202 °C. ^{1}H NMR

(200 MHz, $CDCl_3$): $\delta = 2.46-2.68$ (2H, m, CH_2), 2.50–2.60 (2H, m, CH_2–N), 5.12 (1H, br s, NH), 5.94 (1H, t, *J* 8.2 Hz, C*H*–Ph) 6.92–7.08 (3H, m, Ar-H), 7.28–7.38 (3H, m, Ar-H), 7.52–7.61 (5H, m, Ar-H). IR (KBr): 3469 (N–H), 2928 (C–H), 2228(C≡N), 1606(C=N) cm^{-1}. LSIMS *m/z*: 419 (MH^+). Anal. calcd. for $C_{24}H_{17}F_3N_4$: C, 68.89; H, 4.09; N, 13.39; found: C, 68.71; H, 4.34; N, 13.09%.

*6.1.1.15. 4-Methyl-4,10-diphenyl-8-(trifluoromethyl)-1,2,3,4-tetrahydropyrimido[1,2-b]indazole-7-carbonitrile (**4g**).* Yield: 660 mg (93%); yellow coloured solid; mp 230 °C. ^1H NMR (200 MHz, $CDCl_3$): $\delta = 2.04$ (3H, s, CH_3), 2.30–2.44 (1H, m, C–CH_2), 2.54–2.62 (1H, m, C–CH_2), 3.12–3.17 (1H, m, CH_2–N), 3.40–3.60(1H, m, CH_2–N), 5.62 (1H, br s, N*H*), 6.90–7.06 (3H, m, Ar-H), 7.22–7.36 (3H, m, Ar-H), 7.50–7.63 (5H, m, Ar-H). IR (KBr): 3397 (N–H), 2929 (C–H), 2220 (C≡N), 1607 (C=N) cm^{-1}. ESIMS *m/z*: 433 (MH^+). Anal. calcd. for $C_{25}H_{19}F_3N_4$: C, 69.43; H, 4.42; N, 12.95; found: C, 69.22; H, 4.23; N, 13.13%.

*6.1.1.16. 4-Methyl-10-phenyl-8-(trifluoromethyl)-4-vinyl-1,2,3,4-tetrahydropyrimido[1,2-b]indazole-7-carbonitrile (**4h**).* Yield: 0.50 g (79%); yellow coloured solid; mp 198 °C. ^1H NMR (200 MHz, $CDCl_3$): $\delta = 1.86$ (3H, s, CH_3–C), 2.1–2.22 (2H, m, CH_2), 3.28–3.38 (2H, m, CH_2–NH), 4.68 (1H, d, *J* 17.8 Hz, HC=CH_2), 5.36 (1H, d, *J* 17.8 Hz, HC=CH_2), 5.42 (1H, br s, N*H*), 5.98–6.18 (1H, m, *H*C=CH_2), 7.02 (1H, m, Ar-H), 7.40–7.6 (5H, m, Ar-H). IR (KBr): 3397 (N–H), 2930 (C–H), 2221 (C≡N), 1609 (C=N). LSIMS *m/z*: 383 (MH^+). Anal. calcd. for $C_{21}H_{17}F_3N_4$: C, 65.96; H, 4.48; N, 14.65; found: C, 66.17; H, 4.66; N, 14.58%.

6.2. Anti-fungal activity:Procedure

Anti-fungal activity is studied by agar cup diffusion method. The ready-made potato dextrose agar (PDA) medium (Himedia, 39 g) was suspended in distilled water (1000 mL) and heated to boiling until it dissolved completely, the medium and petri dishes were autoclaved at pressure of 15 $lb/inch^2$ for 20 min. Agar cup bioassay was employed for testing anti-fungal activity. The medium was poured into sterile petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 mL of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving the compound in DMSO and different concentrations were made (100–150 μg/mL). After inoculation, cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup, different concentrations of test solutions 100–150 μg/mL) were added. Controls were maintained with DMSO and amphotericin-B (50 μg/mL). The treated and the controls were kept at 28 °C for 48 h. Inhibition zones were measured and the diameters were calculated in millimeters. Three to four replicates were maintained for each treatment. Media potato dextrose broth and potato dextrose agar were procured from M/s Himedia Laboratories, Mumbai.

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