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RESEARCH ARTICLE

Synthesis, spectral analysis and *in vitro* microbiological evaluation of novel ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates and 4,5-dihydro-6-(napthalen-2-yl)-4-aryl-2*H*-indazol-3-ols

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Abstract

A series of ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8**–**14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21** were synthesised and characterised by their spectroscopic data. *In vitro* microbiological evaluations were carried out for all the newly synthesised compounds **8**–**21** against clinically isolated bacterial and fungal strains. Compounds **9**, **12** and **20** against *Staphylococcus aureus*, **10**, **12**, **20** against *β-haemolytic streptococcus*, **11**, **17** against *Bacillus subtilis*, **12**, **16** and **20** against *Vibreo cholerae*, **13**, **16** against *Escherichia coli*, **13**, **16**, **18**, **19** against *Salmonella typhii*, **12**, **18** against *Shigella flexneri,* **10** against *Salmonella typhii*, **10**, **13**, **17**, **18** against *Aspergillus flavus*, **12**, **17**, **21** against *Aspergillus niger*, **12**, **15**, **17**, **18**, **20** against *Mucor*, *Rhizopus* and *Microsporeum gypsuem* exhibit potent antimicrobial activity.

Keywords: Ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates, 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2H-indazol-3-ols, hydrazine hydrate, antibacterial activity, antifungal activity

Introduction

The dramatic increase in antibacterial and antifungal resistance has limited the effectiveness of currently used drugs and represents a health threat. Amongst the five membered heterocycles, fused indazoles are an important class of compounds and are of increased importance due to their therapeutic and pharmacological properties. A variety of structurally diverse indazole nuclei have arisen due to their wide variety of biological properties such as antimicrobial activity [1], inhibitors of protein kinase B/Akt [2], antiprotozoal agents [3], antichagasic activity [3], leishmanocidal activity [3], trypanocidal activity [3], inhibitors of S-adenosyl homocysteine/methylthio adenosine (SAH/MTA) nucleosides [4], potent activator of the nitric oxide receptor [5] and inhibition of platelet aggregation [5].

Figure 1 shows that the indazole nucleus is a widely studied pharmacophoric scaffold that has emerged as a core structural unit of various bioactive molecules [6–10]. Adjudin (A) is currently under phase II human trials as a potential non-hormonal male contraceptive drug containing an indazole nucleus, which acts by blocking the production of sperm in the testes, but without affecting testosterone production [6]. It is an analogue of the chemotherapy drug lonidamine, (B) an indazole-carboxylic acid, which for a long time, has been known to inhibit aerobic glycolysis in cancer cells [7]. 7-Nitroindazole (C) acts as a selective inhibitor for neuronal nitric oxide synthase, a haemoprotein enzyme which converts arginine to citrulline and nitric oxide (NO) in neuronal tissue [8]. YM-348, (*S*)-1-(7-ethyl-1H-furo[2,3-g]indazol-1-yl)propan-2-amine (D) is an

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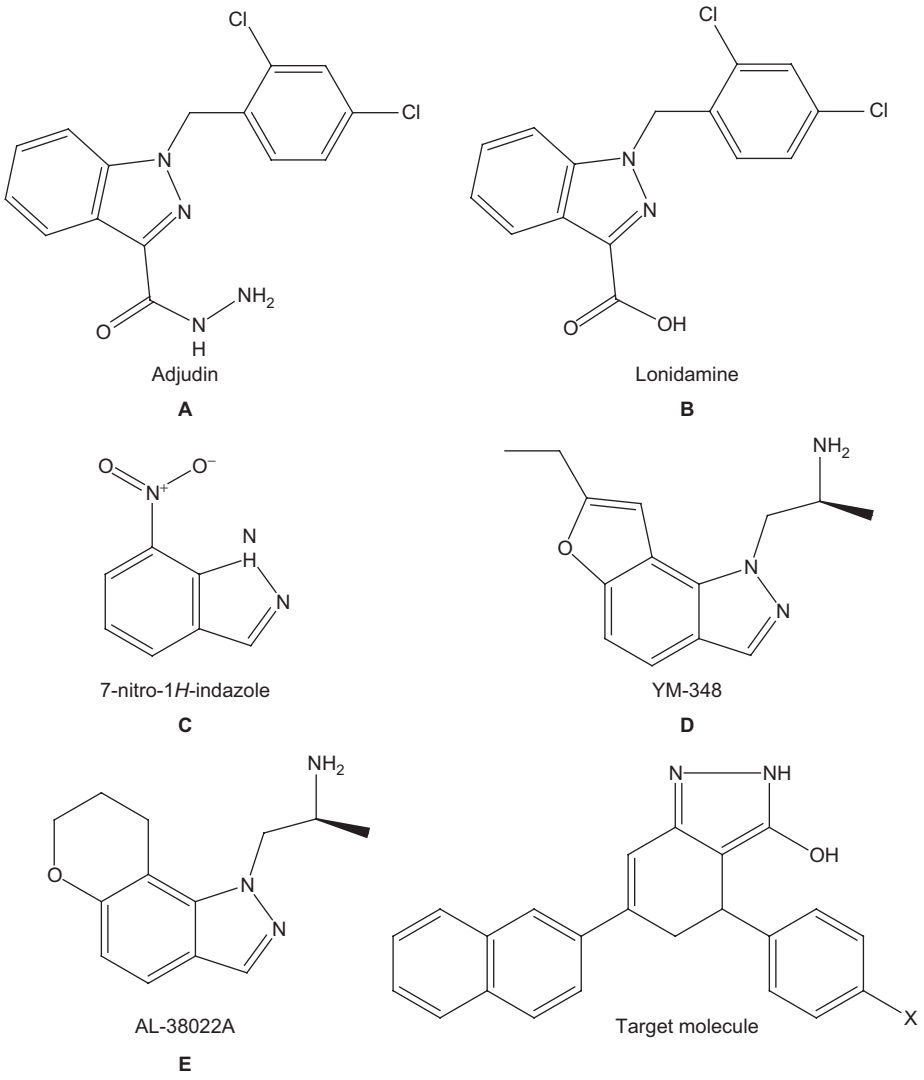


Figure 1. Novel bioactive compounds with a core indazole nucleus of pharmacological importance.

indazole derivative drug which acts as a potent and selective 5-HT_{2C} receptor agonist. It shows thermogenic and anorectic effects in animal studies [9]. AL-38022A, (*S*)-1-(8,9-dihydropyrano[2,3-g]indazol-1(7H)-yl)-propan-2-amine (E) is an indazole derivative drug that acts as a potent and selective agonist for the 5-HT_2 family of serotonin receptors.

A literature survey revealed the value of chalcones as potent biologically active compounds. In recent years there has been a great deal of interest in exploiting more than one proximal functional group for designing novel structures capable of performing a variety of functions. Taking these considerations into account and as part of our research programme aimed at the synthesis of bioactive novel structurally diverse heterocycles [11–15], we report the molecular conjugation of the naphthyl substituted chalcone moiety with two or more active counterparts that has been designed and synthesised with the hope of producing novel ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates (**8**–**14**) an intermediate with three versatile functional groups i.e. ketone, olefin and ester for the synthesis of 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols (**15**–**21**), a novel fused indazole derivative and to study their biopotential against clinically isolated bacterial and fungal strains.

Experimental

Chemistry

The progress of the reaction was monitored by TLC analysis. All the reported melting points are taken in open capillaries and are uncorrected. IR spectra were recorded in KBr (pellet forms) on a Thermo Nicolet-Avatar–330 (Thermo Fisher Scientific Inc., Waltham, US) FT-IR spectrophotometer and important absorption values (cm^{-1}) alone are listed. ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively on a Bruker Avance II 400 NMR spectrometer using DMSO-d_6 as solvent. Two dimensional homonuclear correalation (HOMOCOR) and heteronuclear single quantum correlation (HSQC) spectra were recorded at Bruker DRX 500 (Bruker Biospin International, Ag, Aegeristrasse, Switzerland) NMR spectrometer. The ESI +ve MS spectra

were recorded on a Bruker Daltonics LC-MS spectrometer. Satisfactory microanalyses are obtained on Carlo Erba 1106 (Thermo Fisher Scientific Inc, Waltham, US) CHN analyser. (*E*)-1-naphthalen-2-yl)-3-arylprop-2-en-1-ones **1-7** were prepared according to the literature precedent [16].

General procedure for the synthesis of ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates 8–14

To a solution of sodium ethoxide (0.001 mol) in 30 mL of absolute ethanol, freshly distilled ethyl acetoacetate (0.01 mol) and respective (*E*)-1-naphthalen-2-yl)-3-arylprop-2-en-1-ones (0.01 mol) in absolute ethanol (40mL) was mixed. This mixture was refluxed in a water bath for 1–3 h by maintaining the temperature around 70 to 80°C. The reaction mixture was allowed to cool and filtered. Then the crude product was recrystallised from absolute ethanol to afford ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8–14.**

Ethyl 4-(naphthalen-2-yl)-2-oxo-6-phenylcyclohex-3-enecarboxylate 8

IR (KBr) ν (cm^{-1}): 3057, 2987, 2929, 1738, 1659, 1606, 1292, 1155, 847, 816, 766, 704; ^{1}H NMR (δ ppm), (*J* Hz): 0.92 (3H, t, CH_2CH_3 at C-1, *J* = 7.1), 3.22-3.28 (2H, H_5, m), 3.65–3.73 (1H, H_6, m), 3.91 (2H, q, CH_2CH_3 at C-1, *J* = 7), 4.17 (1H, H_1, d, *J* = 13.4), 6.73 (1H, s, H_3), 7.24–8.33 (12H, m, H_{arom}.); ^{13}C NMR (δ ppm): 13.7 CH_2CH_3 at C-1, 35.1 C-5, 43.8 C-6, 58.7 CH_2CH_3 at C-1, 59.9 C-1, 123.1 C-3, 169.2 C=O at C-1, 194.2 C-2, 123.4-128.8 -C_{arom}., C-4 carbon may be merged with -C_{arom}, 132.7, 133.6, 134.3, 141.5 *ipso*-C's.

Ethyl 4-(naphthalen-2-yl)-2-oxo-6-p-tolylcyclohex-3-enecarboxylate 9

IR (KBr) ν (cm^{-1}): 3065, 3027, 2979, 2923, 2863, 1740, 1660, 1604, 1148, 897, 849, 816, 753; ^{1}H NMR (δ ppm), (*J* Hz): 0.96 (3H, t, CH_2CH_3 at C-1, *J* = 7.1), 2.28 (3H, s, CH_3 at phenyl ring), 3.15–3.17 (2H, H_5, m), 3.62-3.66 (1H, H_6, m), 3.92 (2H, q, CH_2CH_3 at C-1, *J* = 6.9), 4.11 (1H, H_1, d, *J* = 13.3), 6.71 (1H, s, H_3), 7.13–8.30 (11H, m, H_{arom}.); ^{13}C NMR (δ ppm): 14.2 CH_2CH_3 at C-1, 21 CH_3 at phenyl ring, 35.7 C-5, 43.8 C-6, 59.2 CH_2CH_3 at C-1, 60.3 C-1, 123.5 C-3, 169.6 C=O at C-1, 194.7 C-2, 123.7-129.2 -C_{arom}., C-4 carbon may be merged with -C_{arom}, 133.1, 134, 134.7, 136.5, 138.9 *ipso*-Cs.

Ethyl 6-(4-fluorophenyl)-4-(naphthalen-2-yl)-2-oxocyclohex-3-enecarboxylate 10

IR (KBr) ν (cm^{-1}): 3054, 2989, 2925, 1739, 1656, 1603, 1225, 1153, 888, 841, 815, 752; ^{1}H NMR (δ ppm), (*J* Hz): 0.94 (3H, t, CH_2CH_3 at C-1, *J* = 7), 3.19–3.21 (2H, H_5, m), 3.68–3.75 (1H, H_6, m), 3.93 (2H, q, CH_2CH_3 at C-1, *J* = 7), 4.16 (1H, H_1, d, *J* = 13.4), 6.73 (1H, s, H_3), 7.16–8.33 (11H, m, H_{arom}.); ^{13}C NMR (δ ppm): 13.8 CH_2CH_3 at C-1, 35 C-5, 43 C-6, 58.8 CH_2CH_3 at C-1, 59.9 C-1, 123 C-3, 169.1 C=O at C-1, 194.1 C-2, 114.9-129.6 -C_{arom}., C-4 carbon may be merged with -C_{arom}, 132.7, 1333.6, 134.2, 137.7, 160 *ipso*-Cs.

Ethyl 6-(4-methoxyphenyl)-4-(naphthalen-2-yl)-2-oxocyclohex-3-enecarboxylate 11

IR (KBr) ν (cm^{-1}): 3056, 2987, 2932, 2838, 1738, 1657, 1606, 1252, 888, 831, 815, 749; ^{1}H NMR (δ ppm), (*J* Hz): 0.96 (3H, t, CH_2CH_3 at C-1, *J* = 7), 3.16–3.29 (2H, H_5, m), 3.59–3.67 (1H, H_6, m), 3.73 (3H, s, OCH_3 at phenyl ring), 3.92 (2H, q, CH_2CH_3 at C-1, *J* = 7), 4.10 (1H, H_1, d, *J* = 14.3), 6.71 (1H, s, H_3), 6.89–8.32 (11H, m, H_{arom}.); ^{13}C NMR (δ ppm): 14.2 CH_2CH_3 at C-1, 35.7 C-5, 43.5 C-6, 55.4 OCH_3 at phenyl ring, 59.5 CH_2CH_3 at C-1, 60.2 C-1, 123.5 C-3, 169.7 C=O at C-1, 194.7 C-2, 114.1–129 -C_{arom}., C-4 carbon may be merged with -C_{arom}, 129.2, 133.1, 134, 134.7, 159.1 *ipso*-Cs.

Ethyl 6-(4-chlorophenyl)-4-(naphthalen-2-yl)-2-oxocyclohex-3-enecarboxylate 12

IR (KBr) ν (cm^{-1}): 3056, 3022, 2978, 2928, 1738, 1658, 1257, 1147, 890, 850, 818, 749; ^{1}H NMR (δ ppm), (*J* Hz): 0.95 (3H, t, CH_2CH_3 at C-1, *J* = 7), 3.19-3.29 (2H, H_5, m), 3.68-3.75 (1H, H_6, m), 3.93 (2H, q, CH_2CH_3 at C-1, *J* = 7.1), 4.18 (1H, H_1, d, *J* = 13.4), 6.73 (1H, s, H_3), 7.4–8.32 (11H, m, H_{arom}.); ^{13}C NMR (δ ppm): 13.8 CH_2CH_3 at C-1, 34.8 C-5, 43.1 C-6, 58.5 CH_2CH_3 at C-1, 60 C-1, 123 C-3, 169.1 C=O at C-1, 193.9 C-2, 123.3-129.5 -C_{arom}., C-4 carbon may be merged with -C_{arom}, 132.7, 133.6, 134.2, 134.9, 140.5 *ipso*-Cs.

Ethyl 6-(4-bromophenyl)-4-(naphthalen-2-yl)-2-oxocyclohex-3-enecarboxylate 13

IR (KBr) ν (cm^{-1}): 3058, 3027, 2977, 2929, 2907, 2863, 1732, 1657, 1598, 1170, 1145, 892, 817, 748, 711; ^{1}H NMR (δ ppm), (*J* Hz): 0.96 (3H, t, CH_2CH_3 at C-1, *J* = 7), 3.19-3.21 (2H, H_5, m), 3.67–3.74 (1H, H_6, m), 3.94 (2H, q, CH_2CH_3 at C-1, *J* = 7), 4.18 (1H, H_1, d, *J* = 13.4), 6.73 (1H, s, H_3), 7.42–8.32 (11H, m, H_{arom}.); ^{13}C NMR (δ ppm): 13.8 CH_2CH_3 at C-1, 34.8 C-5, 43.2 C-6, 58.5 CH_2CH_3 at C-1, 60 C-1, 123.1 C-3, 169.1 C=O at C-1, 194 C-2, 120.1-129.9 -C_{arom}., C-4 carbon may be merged with -C_{arom}, 131.3, 132.7, 133.6, 134.2, 141 *ipso*-Cs.

Ethyl 4-(naphthalen-2-yl)-6-(4-nitrophenyl)-2-oxo-cyclohex-3-enecarboxylate 14

IR (KBr) ν (cm^{-1}): 3057, 2978, 2925, 2852, 1734, 1658, 1599, 1278, 1149, 893, 856, 815, 743; ^{1}H NMR (δ ppm), (*J* Hz): 0.94 (3H, t, CH_2CH_3 at C-1, *J* = 6.9), 3.17–3.26 (2H, H_5, m), 3.68–3.73 (1H, H_6, m), 3.92 (2H, q, CH_2CH_3 at C-1, *J* = 6.8), 4.17 (1H, H_1, d, *J* = 13.7), 6.73 (1H, s, H_3), 6.98–8.22 (11H, m, H_{arom}.); ^{13}C NMR (δ ppm): 13.5 CH_2CH_3 at C-1, 34.8 C-5, 43.3 C-6, 58.7 CH_2CH_3 at C-1, 60.3 C-1, 123.2 C-3, 169.8 C=O at C-1, 193.2 C-2, 123.8-129.1 -C_{arom}., C-4 carbon may be merged with -C_{arom}, 132.6, 133.6, 134.3, 134.8, 140.1 *ipso*-Cs.

General method for the synthesis of 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2H-indazol-3-ols 15-21

A solution of ethyl 4-(naphthalen-6-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8-14** (0.001 mol) in ethanol (40 mL) was treated with hydrazine hydrate (0.001 mol)

and anhydrous sodium acetate (0.001 mol) and refluxed for 6–8 h. The reaction mixture was cooled and then poured over crushed ice. After filtration, the crude products were recrystallised twice using ethanol as solvent to afford the product.

4,5-dihydro-6-naphthalen-2-yl-4-phenyl-2H-indazol-3-ol 15

IR (KBr) (cm^{-1}): 3399, 3213, 3055, 3016, 2929, 2863, 2728, 1598, 1500, 848, 815, 747, 699; ^{1}H NMR (δ ppm) (*J* Hz) : 3.01–3.09 (1H, m, H_{5a}), 3.25–3.29 (1H, m, H_{5e}), 4.22–4.24 (1H, dd, $J_{4a,5a}$=8.1 Hz, $J_{4a,5e}$=3.2Hz), 6.94 (1H, s), 7.08–8.26 (12H, m, H_{arom}.), 9.59 (1H, bs, H_2), 11.61 (1H, bs, H_3); In the D_2O exchanged ^{1}H NMR spectrum, two broad singlets, one at 9.59 ppm due to labile NH proton and another at 11.61 ppm due to OH proton disappeared; ^{13}C NMR (δ ppm): 33.9 C-4, 36.1 C-5, 98.6 C-9, 113.8 C-7, 142.2 C-8, 158.2 C-3, 123.3-128.5 C_{arom}., C-6 carbon may be merged with C_{arom}., 132.2, 133, 134.7, 135.8, 137.2, 141.2 *ipso*-Cs.

4,5-dihydro-6-naphthalen-2-yl-4-p-tolyl-2H-indazol-3-ol 16

IR (KBr) (cm^{-1}): 3388, 3196, 3050, 3022, 2919, 2858, 2721, 1596, 1509, 848, 813, 772, 745, 670; ^{1}H NMR (δ ppm) (*J* Hz) : 2.18 (3H, s, CH_3 at phenyl ring, 3.01-3.06 (1H, m, H_{5a}), 3.21–3.28 (1H, m, H_{5e}), 4.17-4.2 (1H, dd, $J_{4a,5a}$=8.3 Hz, $J_{4a,5e}$=3.6Hz), 6.98 (1H, s), 7–7.96 (11H, m, H_{arom}.), 9.65 (1H, bs, H_2), 11.7 (1H, bs, H_3); In the D_2O exchanged ^{1}H NMR spectrum, two broad singlets, one at 9.65 ppm due to labile NH proton and another at 11.70 ppm due to OH proton disappeared; ^{13}C NMR (δ ppm): 33.9 C-4, 36.1 C-5, 98.6 C-9, 113.8 C-7, 142.2, C-8, 158.3 C-3, 123.3-128.5 C_{arom}., C-6 carbon may be merged with C_{arom}., 132.2, 133.0, 134.7, 135.8, 137.2, 141.2 *ipso*-Cs.

4-(4-fluorophenyl)-4,5-dihydro-6-(naphthalen-2-yl)-2H-indazol-3-ol 17

IR (KBr) (cm^{-1}): 3382, 3213, 3051, 3022, 2919, 2858, 2721, 1596, 1510, 848, 814, 773, 745, 671; ^{1}H NMR (δ ppm) (*J* Hz) : 3.01–3.09 (1H, m, H_{5a}), 3.21–3.28 (1H, m, H_{5e}), 4.17–4.2 (1H, dd, $J_{4a,5a}$=8.3 Hz, $J_{4a,5e}$=3.6Hz), 6.98 (1H, s), 7–7.96 (11H, m, H_{arom}.), 9.85 (1H, bs, H_2), 11.48 (1H, bs, H_3); In the D_2O exchanged ^{1}H NMR spectrum, two broad singlets, one at 9.85 ppm due to labile NH proton and another at 11.48 ppm due to OH proton disappeared; ^{13}C NMR (δ ppm): 33.9 C-4, 36.1 C-5, 98.6 C-9, 113.8 C-7, 142.2, C-8, 158.3 C-3, 123.3-128.5 C_{arom}., C-6 carbon may be merged with C_{arom}., 133, 135, 135.8, 137.2, 141, 156.4 *ipso*-Cs.

4,5-dihydro-4-(4-methoxyphenyl)-6-(naphthalene-2-yl)-2H-indazol-3-ol 18

IR (KBr) (cm^{-1}): 3380, 3172, 3053, 2994, 2931, 2891, 2832, 1604, 1508, 817, 746, 774, 705, 673; ^{1}H NMR (δ ppm) (*J* Hz) : 3.01–3.06 (1H, m, H_{5a}), 3.2–3.27 (1H, m, H_{5e}), 3.64 (3H, s, OCH_3 at phenyl ring, 4.16–4.19 (1H, dd, $J_{4a,5a}$=8.2 Hz, $J_{4a,5e}$=3.6Hz), 6.74 (1H, s), 6.76–7.97 (11H, m, H_{arom}.), 9.86 (1H, bs, H_2), 11.34 (1H, bs, H_3); In the D_2O exchanged ^{1}H NMR spectrum, two broad singlets, one at 9.86 ppm due to labile NH proton and another at 11.34 ppm due to OH proton disappeared; ^{13}C NMR (δ ppm): 33.5 C-4, 36.2 C-5, 55 OCH_3 at phenyl ring, 98.8 C-9, 113.8 C-7, 141, C-8, 157.5 C-3, 113.4-129.2 C_{arom}., C-6 carbon may be merged with C_{arom}., 132.2, 133, 135.8, 137.2, 160.1 *ipso*-Cs.

4-(4-chlorophenyl)-4,5-dihydro-6-(naphthalen-2-yl)-2H-indazol-3-ol 19

IR (KBr) (cm^{-1}): 3415, 3180, 3053, 2967, 2925, 2874, 2716, 2574, 1597, 1489, 815, 848, 744, 670; ^{1}H NMR (δ ppm) (*J* Hz) : 3.01–3.06 (1H, m, H_{5a}), 3.23–3.28 (1H, m, H_{5e}), 4.22-4.26 (1H, dd, $J_{4a,5a}$=8.3 Hz, $J_{4a,5e}$=3.9Hz), 6.94 (1H, s), 7.18-7.9 (11H, m, H_{arom}.), 9.9 (1H, bs, H_2), 11.49 (1H, bs, H_3); In the D_2O exchanged ^{1}H NMR spectrum, two broad singlets, one at 9.9 ppm due to labile NH proton and another at 11.49 ppm due to OH proton disappeared; ^{13}C NMR (δ ppm): 33.8 C-4, 35.8 C-5, 97.9 C-9, 113.8 C-7, 144.2, C-8, 156.3 C-3, 123.3-129.1 C_{arom}., C-6 carbon may be merged with C_{arom}., 130.4, 132.3, 133, 135.7, 137 *ipso*-Cs.

4-(4-bromophenyl)-4,5-dihydro-6-(naphthalen-2-yl)-2H-indazol-3-ol 20

IR (KBr) (cm^{-1}): 3355, 3180, 3052, 2924, 2869, 2732, 1596, 1536, 849, 815, 745, 667; ^{1}H NMR (δ ppm) (*J* Hz): 3–3.05 (1H, m, H_{5a}), 3.23–3.27 (1H, m, H_{5e}), 4.21–4.24 (1H, dd, $J_{4a,5a}$=8.2, $J_{4a,5e}$=4), 6.94 (1H, s), 7.12–7.96 (11H, m, H_{arom}.), 9.84 (1H, bs, H_2), 11.57 (1H, bs, H_3); In the D_2O exchanged ^{1}H NMR spectrum, two broad singlets, one at 9.84 ppm due to labile NH proton and another at 11.57 ppm due to OH proton disappeared; ^{13}C NMR (δ ppm): 33.9 C-4, 35.8 C-5, 97.8 C-9, 113.9 C-7, 144.6, C-8, 158.1 C-3, 118.9-129.5 C_{arom}., C-6 carbon may be merged with C_{arom}., 130.3, 130.9, 131.1, 132.3, 133, 135.7, 137 *ipso*-Cs.

4,5-dihydro-6-naphthalen-2-yl-4-(4-nitrophenyl)-2H-indazol-3-ol 21

IR (KBr) (cm^{-1}): 3371, 3191, 3054, 2922, 2847, 1598, 1522, 851, 813, 740, 684; ^{1}H NMR (δ ppm) (*J* Hz): 3.01–3.06 (1H, m, H_{5a}), 3.23–3.28 (1H, m, H_{5e}), 4.21–4.24 (1H, dd, $J_{4a,5a}$=8.1, $J_{4a,5e}$=3.8), 6.93 (1H, s), 6.96–7.88 (11H, m, H_{arom}.), 9.72 (1H, bs, H_2), 11.52 (1H, bs, H_3); In the D_2O exchanged ^{1}H NMR spectrum, two broad singlets, one at 9.72 ppm due to labile NH proton and another at 11.52 ppm due to OH proton disappeared; ^{13}C NMR (δ ppm): 33.7 C-4, 35.6 C-5, 97.6 C-9, 113.8 C-7, 144.5, C-8, 158.4 C-3, 124.9-128.9 C_{arom}., C-6 carbon may be merged with C_{arom}., 130.6, 130.9, 131.1, 132.6, 133.2, 135.6, 137.2 *ipso*-Cs.

Microbiology

Materials

All the clinically isolated bacterial strains namely *Staphylococcus aureus*, β-*haemolytic streptococcus*, *Bacillus subtilis*, *Vibreo cholerae*, *Escherichia coli*, *Salmonella typhii*, *Shigella flexneri* and the fungal strains namely *Aspergillus flavus*, *Aspergillus niger*, *Mucor*, *Rhizopus* and *Microsporeum gypsuem* were obtained from the Faculty of Medicine, Annamalai University, Annamalainagar, Tamil Nadu, India.

In vitro antibacterial and antifungal activity

Minimum inhibitory concentration (MIC) in μg/mL values was carried out by the two-fold serial dilution method [17]. The respective test compounds (**8**–**21**) were dissolved in dimethyl sulphoxide (DMSO) to obtain a 1 mg mL^{-1} stock solution. Seeded broth (broth containing microbial spores) was prepared in nutrient broth (NB) from 24 h old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C while fungal spores from 1 to 7 day old Sabourauds agar (Hi-media, Mumbai) slant cultures were suspended in Sabouraud's dextrose broth (SDB). The colony forming units (CFU) of the seeded broth were determined by a plating technique and adjusted in the range of 10^4-10^5 CFU/mL. The size of the final inoculums were 10^5CFU/mL for the antibacterial assay and $1.1–1.5 \times 10^2$ CFU/mL for the antifungal assay. Testing was performed at pH 7.4 ± 0.2 for the bacteria (NB) and at a pH 5.6 for the fungi (SDB). Exactly 0.4 mL of the solution of test compound was added to 1.6 mL of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth were kept as controls. The tubes were incubated in biological oxygen demand (BOD) incubators (Sigma Instruments, Chennai, India) at 37 ± 1°C for bacteria and 28 ± 1°C for the fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Ciprofloxacin was used as the standard for bacterial studies and Fluconazole was the standard for fungal studies.

Results and Discussion

Chemistry

The conventional approach for the synthesis of 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15-21** is as follows: (*E*)-1-(naphthalen-2-yl)-3-arylprop-2-ene-1-ones **1-7** are synthesised by the Claisen-Schmidt condensation of 2-acetyl naphthalene and substituted benzaldehydes in the presence of alcoholic sodium hydroxide. Treatment of (*E*)-1-(naphthalen-2-yl)-3-arylprop-2-ene-1-ones **1**–**7** with ethyl acetoacetate in the presence of sodium ethoxide in refluxing ethanol (Scheme 1 and Table 1) afford ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8–14.** Compounds **8**–**14** on reaction with hydrazine hydrate in the presence of anhydrous sodium acetate in refluxing ethanol yield the respective indazole derivatives, 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21**. The structures of all the newly synthesised ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8**–**14** were confirmed by FT-IR, MS, ^{1}H NMR and ^{13}C NMR spectral studies and elemental analysis. Moreover, 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21** were confirmed by mps, FT-IR, MS, ^{1}H NMR, D_2O exchanged ^{1}H NMR, ^{13}C NMR, two dimensional ^{1}H-^{1}H HOMOCOR and ^{1}H-^{13}C HSQC spectral studies and elemental analysis.

The reaction mechanism for the formation of ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8**–**14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21** is shown in Scheme 2.

In order to discuss the spectral data of Ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8**–**14**, ethyl 4-(naphthalen-2-yl)-2-oxo-6-phenyl cyclohex-3-enecarboxylate **8** has been chosen as a representative compound.

Analysis of FT-IR spectrum of ethyl 4-(naphthalen-2-yl)-2-oxo-6-phenylcyclohex-3-enecarboxylate 8:

FT-IR spectrum of ethyl 4-(naphthalen-2-yl)-2-oxo-6-phenylcyclohex-3-enecarboxylate **8** shows two strong characteristic absorptions at 1738 and 1659 cm^{-1} due to the ester carbonyl and ketone functional groups respectively. The band at 1606 cm^{-1} is due to the presence of C=C stretching frequency. The absorption frequency at 3057 cm^{-1} is assigned to aromatic C-H stretching vibration and the absorption frequencies at 2987 and 2929 cm^{-1} is assigned to aliphatic C-H stretching vibration. The observed ester carbonyl, ketone and C=C stretching vibrational bands are supporting evidence for the formation of synthesised compound **8**.

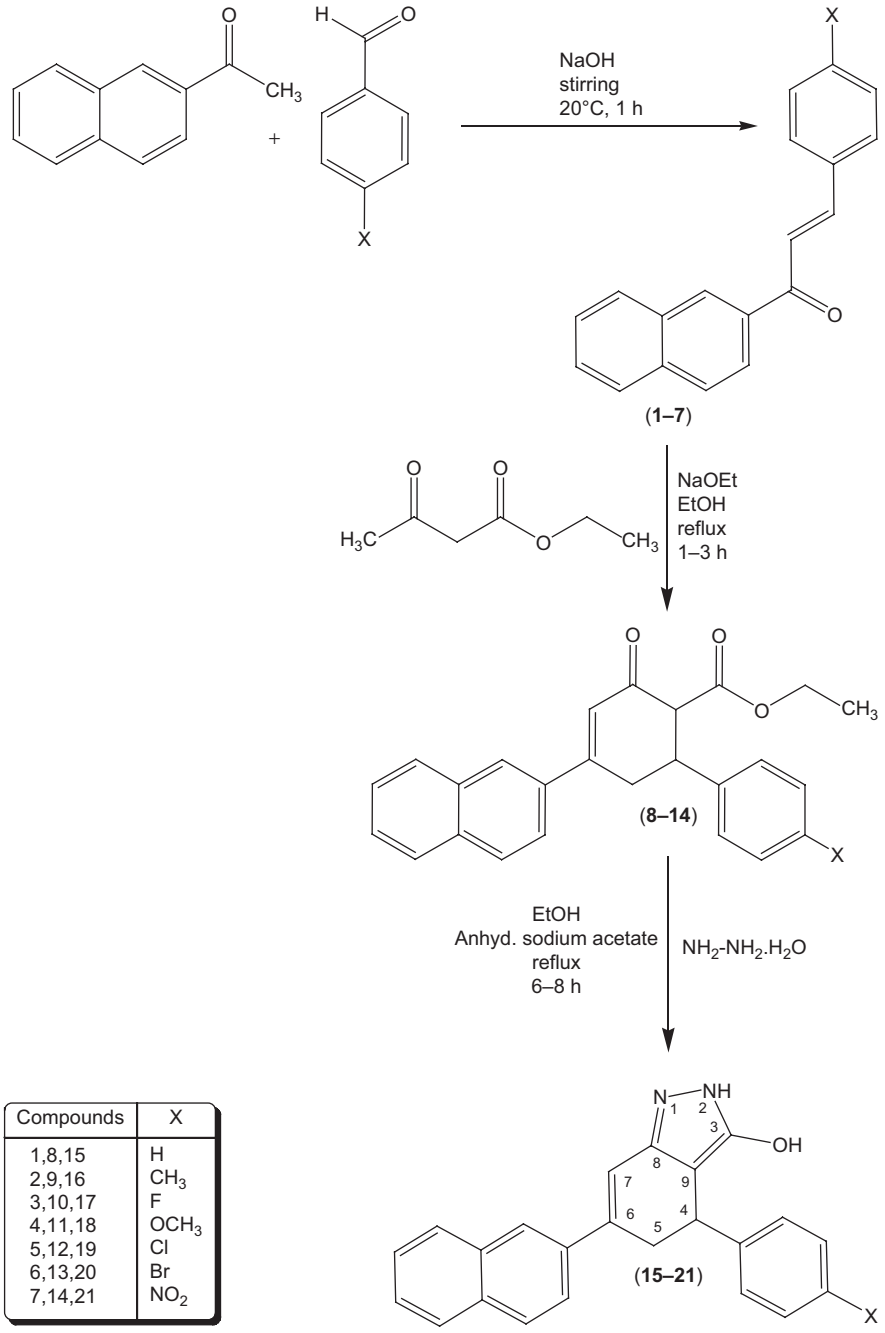
Analysis of ^{1}H NMR spectrum of ethyl 4-(naphthalen-2-yl)-2-oxo-6-phenylcyclohex-3-enecarboxylate 8:

In the ^{1}H NMR spectrum of **8**, a triplet was observed at 0.92 ppm (J = 7.1 Hz) corresponding to three protons and this signal is due to the ester methyl protons at C-1. A quartet was observed at 3.91 ppm (J = 7 Hz) corresponding to two protons and this signal was due to the ester methylene protons at C-1. Two multiplets are obtained in the range 3.22–3.28 and 3.65–3.73 and they are due to H-5 and H-6 protons. The doublet at 4.17ppm (J = 13.4 Hz) has been assigned to the H-1 proton. The singlet observed in downfield region at 6.73 ppm is due to the H-3 proton. The aromatic protons appeared as a multiplet in the range 7.24–8.33ppm.

Analysis of ^{13}C NMR spectrum of ethyl 4-(naphthalen-2-yl)-2-oxo-6-phenylcyclohex-3-enecarboxylate 8

Two ^{13}C resonances at 194.2 and 169.26 ppm are assigned to the C-2 carbonyl carbon and ester carbonyl carbon respectively. The ^{13}C resonances at 35.1 and 43.8 ppm are due to the C-5 and C-6 carbons respectively. The ^{13}C resonance observed at 58.7 and 13.7 ppm were assigned to the ester methylene and methyl carbons at C-1 respectively. The signal observed at 59.9 ppm is assigned to the C-1 carbon, whereas the signal at 123.1 ppm is assigned to the C-3 carbon. The aromatic carbons are observed in the range of 123.4–128.8 ppm. C-4 carbon may be merged with the aromatic carbons. The remaining ^{13}C signals at 132.7, 133.6, 134.3, 141.5 ppm were due to *ipso* carbons.

In order to discuss the spectral data of 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21**, 4,5-dihydro-6-naphthalen-2-yl-4-phenyl-2*H*-indazol-3-ol **15** was chosen as the representative compound.



Compounds	X
1,8,15	H
2,9,16	CH_3
3,10,17	F
4,11,18	OCH_3
5,12,19	Cl
6,13,20	Br
7,14,21	NO_2

Scheme 1. Synthetic route for the formation of new ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8**–**14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21**.

Analysis of FT-IR spectrum of 4,5-dihydro-6-naphthalen-2-yl-4-phenyl-2H-indazol-3-ol 15

The FT-IR spectrum of 4,5-dihydro-6-naphthalen-2-yl-4-phenyl-2*H*-indazol-3-ol **15** shows two characteristic absorption frequencies at 3399 and 3213 cm^{-1} suggests the presence of -OH and -NH functional groups. The absorption frequency at 1598 cm^{-1} is assigned to C=N stretching vibration. The band at 1500 cm^{-1} is due to the presence of C=C stretching frequency. Besides these, aromatic CH stretching frequencies were observed at 3055 and 3016 cm^{-1} and the aliphatic CH stretching frequencies were observed at 2929, 2863, and 2728 cm^{-1}. The observed –OH, -NH, C=N, C=C stretching vibrational bands are supporting evidence for the formation of the synthesised compound **15**.

Analysis of ^{1}H NMR spectrum of 4,5-dihydro-6-naphthalen-2-yl-4-phenyl-2H-indazol-3-ol 15

In the ^{1}H NMR spectrum of compound **15**, a doublet of doublet was observed for the H-4 proton and two coupling constants were extracted from it, namely $J_{4a,5a}$ = 8.1 Hz and $J_{4a,5e}$ = 3.2 Hz. Two multiplets in the region 3.01–3.09 and 3.25–3.29 ppm were assigned to H_{5a} and H_{5e} respectively. A singlet at 6.94 ppm was conveniently assigned to the H-7 proton. The aromatic protons appeared as a multiplet around 7.08–8.26 ppm. The labile OH and NH protons

Table 1. Physical and analytical data of ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8**–**14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21**.

					Elemental analysis			$m/z\ (M+1)^{+\bullet}$ Molecular formula
Entry	X	Reaction time(h)	Yield(%)	m.p.(°C)	CFound(Calculated)	HFound(Calculated)	NFound(Calculated)	
8	H	2	80	146	81.01 (81.06)	5.92 (5.99)	-	$371 C_{25}H_{22}O_3$
9	CH_3	3	78	110	81.17 (81.22)	6.25 (6.29)	-	$385 C_{26}H_{24}O_3$
10	F	1	82	126	77.24 (77.3)	5.4 (5.45)	-	$389 C_{25}H_{21}FO_3$
11	OCH_3	3	80	122	77.91 (77.98)	6 (6.04)	-	$401 C_{26}H_{24}O_4$
12	Cl	1	75	114	74.12 (74.16)	5.2 (5.23)	-	$405 C_{25}H_{21}ClO_3$
13	Br	1	78	126	66.76 (66.82)	4.67 (4.71)	-	$449 C_{25}H_{21}BrO_3$
14	NO_2	2	65	132	72.22 (72.28)	5.07 (5.1)	3.33 (3.37)	$416 C_{25}H_{21}NO_5$
15	H	8	60	187	81.57 (81.63)	5.29 (5.36)	8.21 (8.28)	$339 C_{23}H_{18}N_2O$
16	CH_3	6	55	173	81.71 (81.79)	5.64 (5.72)	7.9 (7.95)	$353 C_{24}H_{20}N_2O$
17	F	8	62	165	77.44 (77.51)	4.75 (4.81)	7.79 (7.86)	$357 C_{23}H_{17}FN_2O$
18	OCH_3	6	50	176	78.18 (78.24)	5.41 (5.47)	7.54 (7.6)	$369 C_{24}H_{20}N_2O_2$
19	Cl	8	45	180	74.01 (74.09)	4.54 (4.6)	7.46 (7.51)	$373 C_{23}H_{17}ClN_2O$
20	Br	8	60	190	66.13 (66.2)	4.07 (4.11)	6.66 (6.71)	$417 C_{23}H_{17}BrN_2O$
21	NO_2	6	45	172	72 (72.05)	4.41 (4.47)	10.89 (10.96)	$384 C_{23}H_{17}N_3O_3$

(exchangeable with D_2O) appeared as a broad singlet at 11.61 and 9.59 ppm respectively.

Analysis of ^{1}H-^{1}H COSY spectrum of 4,5-dihydro-6-naphthalen-2-yl-4-phenyl-2H-indazol-3-ol 15

In the homonuclear correlation spectroscopy (HOMOCOSY) spectrum of **15**, the signal at 4.22–4.24 ppm showed cross peaks with the signals at 3.01–3.09 and 3.25–3.29 ppm. The signal at 3.25–3.29 ppm showed cross peaks with 3.01–3.09 ppm and 4.22–4.24 ppm. Similarly the signals at 3.01–3.09 showed cross peaks with 3.25–3.29 and 4.22-4.24 ppm. The signal at 4.22-4.24 ppm must be due to the H-4 proton, since this can have coupling with the H_{5a} and H_{5e} protons. Consequently, two signals at 3.01–3.09 ppm and 3.25–3.29 ppm must be due to the H_{5a} and H_{5e} protons. The signal at 6.94 ppm showed a cross peak with 3.25–3.29 ppm and vice versa. Hence the signal at 6.94 ppm was conveniently assigned to the H-7 proton and the signal at 3.25–3.29 ppm was assigned to H_{5e}.

Analysis of ^{13}C NMR spectrum of 4,5-dihydro-6-naphthalen-2-yl-4-phenyl-2H-indazol-3-ol 15

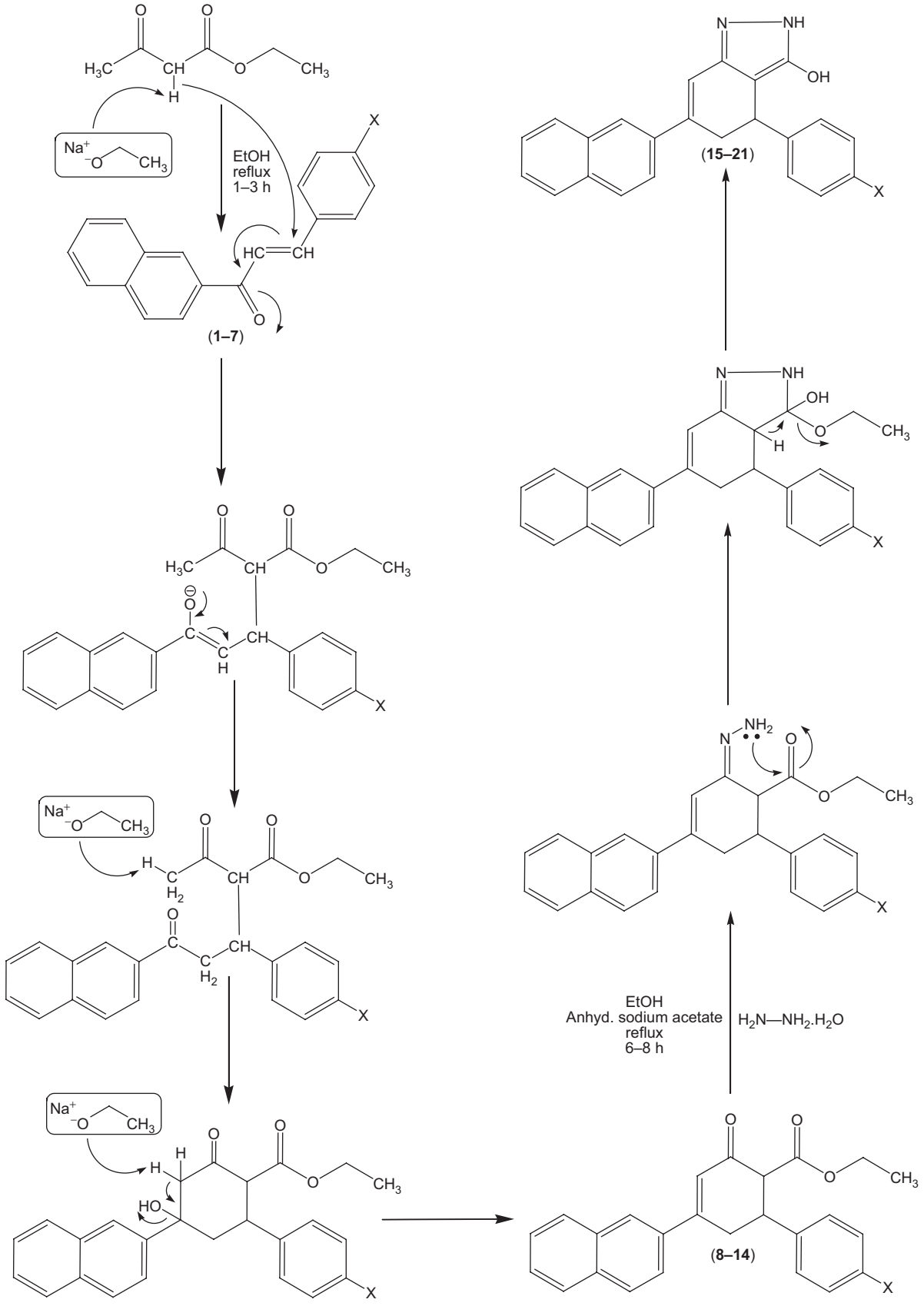
In the ^{13}C NMR spectrum of **15**, the two resonances in the aliphatic region at 34.3 and 35.9 ppm were due to the C-4 and C-5 carbons respectively. The remaining ^{13}C resonances in the quaternary carbon signals at 158.2, 113.8, 145.3 and 98.4 were due to C-3, C-7, C-8 and C-9 carbons. The aromatic carbons were observed in the range of 123.3–129.6 ppm. The ^{13}C resonances at 132.2, 133, 135.8, 137.2 and 138.1 were due to ipso carbons.

Analysis of ^{1}H-^{13}C HSQC spectrum of 4,5-dihydro-6-naphthalen-2-yl-4-phenyl-2H-indazol-3-ol 15

In the HSQC spectrum of **15**, one bond correlation (34.3/4.22–4.24 ppm) was observed between C-4 and H-4. The ^{13}C resonance at 35.9 has correlations with the methylene protons H_{5a} and H_{5e} (35.9/3.01–3.09; 35.9/3.25–3.29) and hence C-5 resonates at 35.9 ppm. The ^{13}C resonance at 113.8 ppm has correlations with singlet at 6.94 ppm. So the signal at 6.94 ppm was conveniently assigned to the H-7 proton and the carbon signal at 113.8 ppm was assigned to C-7.In the HSQC, the ^{13}C resonances at 98.4, 145.3 and 158.2 ppm has no correlations with protons and hence is due to quaternary carbons. The ^{13}C resonances at 98.4, 145.3 and 158.2 ppm were due to the C-9, C-8 and C-3 carbons respectively. The C-6 carbon may be merged with the aromatic carbons. Among the quaternary carbons, the ^{13}C resonances at 132.2, 133.0, 135.8, 137.2, and 138.1 are due to ipso carbons.

Antibacterial activity

Novelethyl4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8**–**14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21** were tested for their antibacterial activity *in vitro* against *S. aureus, β.H. streptococcus, B. subtilis, V. cholerae, E. coli, S. typhii and S. flexneri.* ciprofloxacin was used as the standard drug. Minimum inhibitory concentration (MIC) in μg/mL values is shown in Table 2. A close survey of the MIC values indicates that all the compounds **(8–21)** exhibit a varied range (6.25–200 μg/mL) of antibacterial activity against all the tested bacterial strains except compounds **8**, **15** and **16** against *E. coli, S. aureus* and *β.H. streptococcus* respectively, which didn't have activity even at a maximum concentration of 200 μg/mL. Compound **8** which with no substitution at the *para* position of the phenyl rings attached to C-4 of the cyclohexenone moiety exerted moderate activity against all the tested bacterial strains. Compounds **9** and **11,** which have an electron donating methyl/methoxy substituent at the *para* position of the phenyl rings attached to the C-4 of cyclohexenone moiety, exerted excellent antibacterial activity against the all the gram positive bacterial strains that were tested,



Scheme 2. Reaction mechanism for the formation of title compounds ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates and 4,5-dihydro-6-(napthalen-2-yl)-4-aryl-2*H*-indazol-3-ols.

Table 2. *In vitro* antibacterial activity (MIC) values for ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8**–**14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21**.

	Minimum Inhibitory Concentration (MIC) in μg/mL						
Compound	*S. aureus*	*β-H.streptococcus*	*B. subtilis*	*V. cholerae*	*E. coli*	*S. typhii*	*S. flexneri*
8	*100*	*200*	50	200	-[a]	100	50
9	*6.25*	*12.5*	12.5	100	200	100	100
10	*12.5*	*6.25*	12.5	25	25	6.25	12.5
11	*12.5*	*12.5*	6.25	200	100	100	50
12	*6.25*	*6.25*	12.5	6.25	25	25	6.25
13	*25*	*25*	12.5	25	6.25	25	12.5
14	*50*	*100*	50	100	100	50	50
15	-[a]	*50*	50	100	100	100	200
16	*100*	-[a]	50	6.25	6.25	25	12.5
17	*12.5*	*12.5*	6.25	100	25	12.5	25
18	*200*	*50*	50	25	6.25	12.5	6.25
19	*25*	*25*	50	12.5	6.25	25	12.5
20	*6.25*	*6.25*	12.5	6.25	50	25	25
21	*100*	*50*	50	25	25	25	50
Ciprofloxacin	*25*	*50*	25	50	25	25	25

[a] - No inhibition even at higher concentration i.e. at 200 μg/mL

namely *S. aureus, β.H. streptococcus* and *B. subtilis* at MIC values of 6.25–12.5 μg/mL whereas they exerted only moderate activity against the gram negative bacterial strains. Compounds which contain an electron withdrawing fluoro, chloro or bromo substituent at the *para* position of phenyl rings attached to the C-4 of the cyclohexenone moiety exerted excellent activity against all the tested bacterial strains, except the nitro substituent which possess moderate activity. The novel indazole derivative **15** exerted moderate activity against both gram positive and gram negative bacterial strains, whereas the introduction of a methyl/methoxy substitutent at the *para* position of the phenyl rings attached to the C-4 of the cyclohexenone moiety in compounds **16** and **18** respectively possessed excellent antibacterial activity against al the gram negative bacterial strains tested. Compounds **16** and **18** showed only moderate activity against gram positive bacterial strains and had MIC values in the range of 50–200 μg/mL. With the exception of the nitro substitutent of compound **21**, all the electron withdrawing substituents namely fluoro, chloro or bromo compounds such as **17**, **19** and **20** exerted strong antibacterial activity against all the tested strains when compared to the standard drug ciprofloxacin.

Antifungal activity

The *in vitro* antifungal activity of ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8**–**14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21** was studied against the following fungal strains: *A. flavus, A. niger, Mucor, Rhizopus and Microsporeum gypsuem.* Fluconazole was used as the standard drug. Minimum inhibitory concentration (MIC) in μg/mL values are shown in Table 3. A close survey of the MIC values indicates that compounds **8**–**14** exhibited a varied range (6.25–200 μg/mL) of antifungal activity against all the tested fungal strains except compounds **9**, **11**, **16** and **21** which didn't show antifungal activity against *Rhizopus*, *A. niger, Mucor* and *A. flavus* respectively even at a high concentration of 200 μg/mL. Compound **8**, having no substitution at the phenyl rings attached to the C-4 carbon of cyclohexenone moiety exerted moderate activity against all the tested fungal strains, whereas their indazole derivative compound **15** exerted good antifungal activity against all the tested fungal strains at a MIC value range of 50–6.25 μg/mL. Similar results were observed for the electron donating methyl and methoxy substituent compounds **9** and **11** when compared to their indazole derivative compounds **16** and **18**. Cyclohexenone compounds **10**, **12**, **13** and **14** as well as the indazole compounds **17**, **19, 20** and **21** which all have electron withdrawing fluoro, chloro, bromo or nitro substituents at the *para* position of the phenyl rings attached to C-4 of the cyclohexenone moiety exerted excellent activity against all the fungal strains tested and most of them had MIC values in the range of 25–6.25 μg/mL. All the indazole derivatives **15**–**21** were more potent than their counterpart cyclohexenone ester derivatives **8**–**14** and exerted good antifungal activity when compared to the standard drug fluconazole. Among the cyclohexenone ester derivatives **8**–**14**, compounds having electron withdrawing fluoro, chloro, bromo or nitro substituent at the *para* position of phenyl rings attached to the C–4 of the cyclohexenone moiety exerted excellent activity against all the tested fungal strains.

Conclusion

In brief, a series of ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8**–**14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15**–**21** were synthesised and characterised by their physical and analytical data. This reaction may have wide applicability in building a variety of heterocycles by choosing 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-

Table 3. *In vitro* antifungal activity (MIC) values for ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8–14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15–21**.

	Minimum Inhibitory Concentration (MIC) in μg/mL				
Compound	A. flavus	*A. niger*	*Mucor*	*Rhizopus*	*M. gypsuem*
8	*50*	100	50	50	100
9	*25*	50	25	-[a]	100
10	*6.25*	12.5	12.5	50	50
11	*100*	-[a]	100	200	100
12	*25*	6.25	12.5	6.25	6.25
13	*6.25*	12.5	12.5	25	12.5
14	*50*	100	50	25	25
15	*50*	25	12.5	25	6.25
16	*25*	25	-[a]	25	12.5
17	*6.25*	6.25	12.5	6.25	12.5
18	*6.25*	12.5	25	12.5	6.25
19	*25*	25	12.5	6.25	25
20	*100*	50	25	6.25	12.5
21	-[a]	6.25	25	100	50
Fluconazole	*50*	50	25	25	25

[a] - No inhibition even at higher concentration i.e. at 200 μg/mL

3-enecarboxylates as synthon, which has three versatile functional groups i.e., ketone, olefin and ester for the synthesis of structurally diverse organic compounds. Hence it may be an attractive compound for organic synthesis. The microbiological screening studies carried out to evaluate the antibacterial and antifungal potencies of the newly synthesised ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8–14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15–21** are shown in Table 2 and Table 3. A close inspection of the *in vitro* antibacterial and antifungal activity profile in differently electron donating (CH_3 and OCH_3) and electron withdrawing (-F, -Cl, Br and $-NO_2$) functional group substituted phenyl rings of novel ethyl 4-(naphthalen-2-yl)-2-oxo-6-arylcyclohex-3-enecarboxylates **8–14** and 4,5-dihydro-6-(naphthalen-2-yl)-4-aryl-2*H*-indazol-3-ols **15–21** exerted strong antibacterial activity against all the tested bacterial strains. Compounds **9**, **12** and **20** exerted a four fold increase in activity against *S. aureus* when compared to the standard drug, and showed excellent antibacterial activity at a MIC value of 6.25 μg/mL. The compounds **10**, **12** and **20,** which all have electron withdrawing substituents, exerted eight fold increases in activity when compared to the standard drug, and good antibacterial activity against *β.H. streptococcus* at a MIC value of 6.25μg/mL. Four fold increases in activity was observed for the chloro substituted compound **11** and the fluoro substituted indazole derivative compound **17** exerted good activities against *B. subtilis*. Compounds **12**, **16** and **20** showed potent antibacterial activity against *V. cholerae* at a MIC value of 6.25 μg/mL., Compounds **13**, **16**, **18** and **19** had good activity against *E.coli*, and compound **10** had good activity against *S. typhii* at a MIC value of 6.25 μg/mL, these are four fold increases in activity when compared to the drug, ciprofloxacin. Compounds **12** and **18** exerted good antibacterial activity against *S. flexneri* at a MIC value of 6.25 μg/mL. Results of the anti-fungal activity study showed that the nature of substituents on the phenyl ring namely, the methyl, fluoro, methoxy, chloro, bromo and nitro functions at the *para* positions of the aryl moieties are determinant for the nature and extent of the anti-fungal activity of all the synthesised compounds **8–21** for the fungal strains namely: *A. flavus, A. niger, Mucor, Rhizopus* and *M. gypsuem.* Compounds **10**, **13** and **17,** which all have electron withdrawing substitutents and electron donating methoxy substitutent compound **18** against *A. flavus* exerted eight fold increases in activity when compared to the standard drug fluconazole. Similarly compounds **12**, **17** and **21** exerted eight fold increases in activity against *A. niger* when compared to the standard drug. Moreover, all the electron withdrawing substituent derivatives such as compounds **12**, **15**, **17** and **20** and electron donating methoxy substitutent compound **18** exerted four fold increases in activity against *Mucor*, *Rhizopus* and *M. gypsuem* when compared to the standard drug fluconazole. Results of the antimicrobial activity showed that electron withdrawing substitutents like fluoro, chloro, bromo and nitro substituted derivatives exerted excellent antibacterial and antifungal activities, since electron withdrawing substitutents increase the lipophilicity due to the strong electron withdrawing capability [18]. Moreover, electron withdrawing substitutents namely fluorine substitution was commonly used in contemporary medicinal chemistry to improve metabolic stability, bioavailability and protein ligand interactions [19]. Replacement of the phenyl ring at position 4 in 4,6-diaryl-4,5-dihydro-3-hydroxy-2[*H*]-indazoles [14] by a more liphophilic naphthalene core [20] enhanced the microbiological activity against the tested bacterial and fungal strains. These observations may promote a further

development of our research in this field. Furthermore, the observed marked antibacterial and antifungal activities of this group of naphthyl cyclohexenones and their indazole derivatives may be considered as key steps for the building of novel chemical entities with comparable pharmacological profiles to that of the standard drugs.

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Declaration of Interest

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