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Silica Perchloric acid catalysed synthesis of 2-Benzoyl-6-(3-arylsydnon-4-yl)-4-aryl-2,3a,4,5-tetrahydro-3*H*-indazol-3-ones as antimicrobial agents

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*An efficient and practical procedure for the synthesis of 2-benzoyl-6-(3-arylsydnon-4-yl)-4-aryl-2,3a,4,5-tetrahydro-3H-indazol-3-ones (**4f-o**) using inexpensive silica perchloric acid as heterogenous catalyst is described. The use of catalyst increased the yield of the product. The antimicrobial screening for the title compounds was carried out.*

INTRODUCTION

Sydnones are the mesoionic compounds which possess structural features of considerable interest to medicinal chemists. Their significance lies in their ability to interact electrostatically with two complementary partially charged positions on receptor macromolecules, such as a protein helix. Sydnone derivatives have been reported to show antifungal [1], analgesic [2], diuretic [3], and hypotensive [4] activities. Indazolones [5] are an important class of heterocycles having a number of useful applications. 1,2-dihydroindazol-3-ones are potent 5-lipoxygenase inhibitors [6-7] with various degrees of selectivity. Structure activity relationship (SAR) studies indicated that while N-1, N-2 un-substituted derivatives and N-1 substituted derivatives are orally inactive, N-2-alkyl derivatives are orally active and inhibit both 5-LPO and cycloxygenase (CO). In contrast, N-2-benzyl derivatives are selective for 5-LPO but possesses weak oral activity. 1,2-Dihydro-2-(3′-pyridylmethyl)-3*H*-indazol-3-one is reported to be highly orally active inhibitor of 5-LPO and appears to be useful agent for elucidating *in vivo* roles of leukotrienes [8].

The development of non-toxic, low cost, eco-friendly, recyclable catalyst systems which give high productivity under mild reaction conditions have received much attention in organic synthesis [9]. In recent years, solid supported catalysts [10] have gained much importance due to their economic and environmental benefits, easy work-up procedure, easy separation of catalyst and reuse, their thermal stability, and long catalytic life. Silica supported perchloricacid ($HClO_4$–SiO_2) is a versatile solid supported catalyst known to catalyze numerous organic reactions like acetylation [11], condensation [12], N-t-butoxycarbonylation of amines [13], synthesis of 2,3-unsaturated-O-glucosides and furan diol from 2,3-glycals [14], 2, 3-unsaturated glyco-pyranosides [15], Micheal addition of thiols to electron deficient alkenes [16], esterification of carboxylic acid with alcohols [17] etc.

The above facts made us to club the two biologically active molecules and explore their structure activity relationship (SAR) and further study their antimicrobial potency. In this regard we are presenting the silica perchloric acid catalysed synthesis, spectral characterisation and antimicrobial activities of 2-benzoyl-6-(3-arylsydnon-4-yl)-4-aryl-2,3a,4,5-tetrahydro-3*H*-indazol-3-ones **4f-o**.

RESULTS AND DISCUSSION

The starting compound (*E*)-3-phenyl-1-(3-phenylsydnon-4-yl)-prop-2-en-1-one **2f** was treated with ethylacetoacetate in dry acetone in presence of an. K_2CO_3 to get ethyl-2-oxo-6-phenyl-4-(3-phenyl-sydnon-4-yl)-cyclohex-3-enecarboxylate **3f** [18]. The compound **3f** was then treated with benhzydrazide to get the title compounds **4f**. But the yield of the product was negligible and time required for the reaction to occur was 14 hrs.

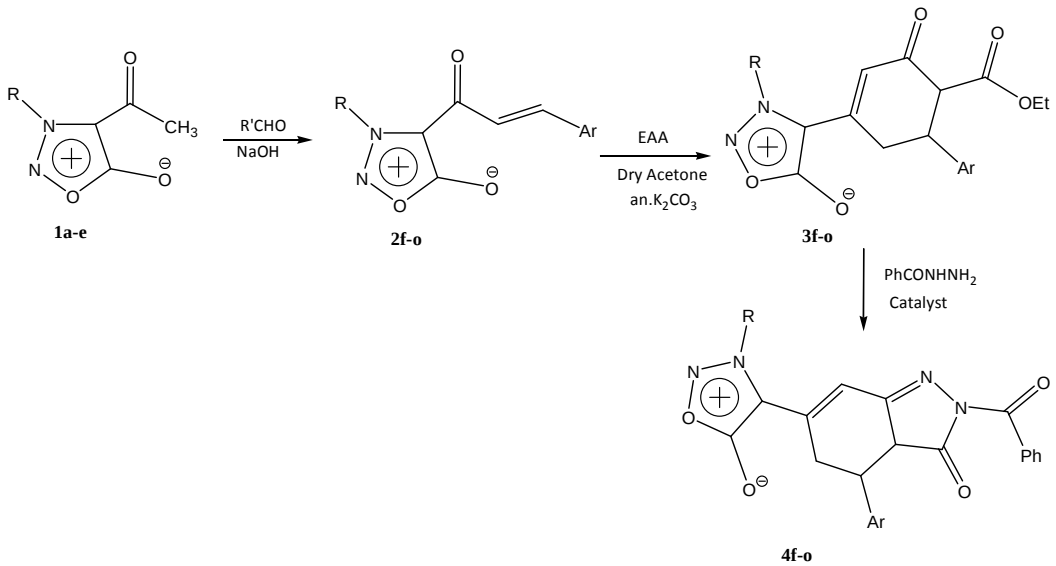
However, in presence of silica perchloric acid (10 mol%) as catalyst the yield of product was increased to 85% (Scheme 1) within 2 hrs. Therefore, all other compounds were prepared using this catalyst. The reason for increased yield is explained by the mechanism given in **Scheme 2**. The yield of the product as well as time required for the completion of the reaction for the compounds **4f-o** are given in the **Table 1**. It was generally observed that the compounds with the electron withdrawing groups required more time for the completion of the reaction and also the yield was comparatively low (compounds **4i**, **4j**, **4n** and **4o**). However, the compounds with electron donating groups required less time and the yield of the product was high.

Table 1. Yield and time required for the completion of the reaction to get the title compounds 4f-o.

Entry No.	Yield (%)	Time (hrs)
4f	85	2.0
4g	86	2.5
4h	82	2.0
4i	75	3.5
4j	70	4.0
4k	82	2.5
4l	80	2.5
4m	81	2.5
4n	74	3.5
4o	70	3.5

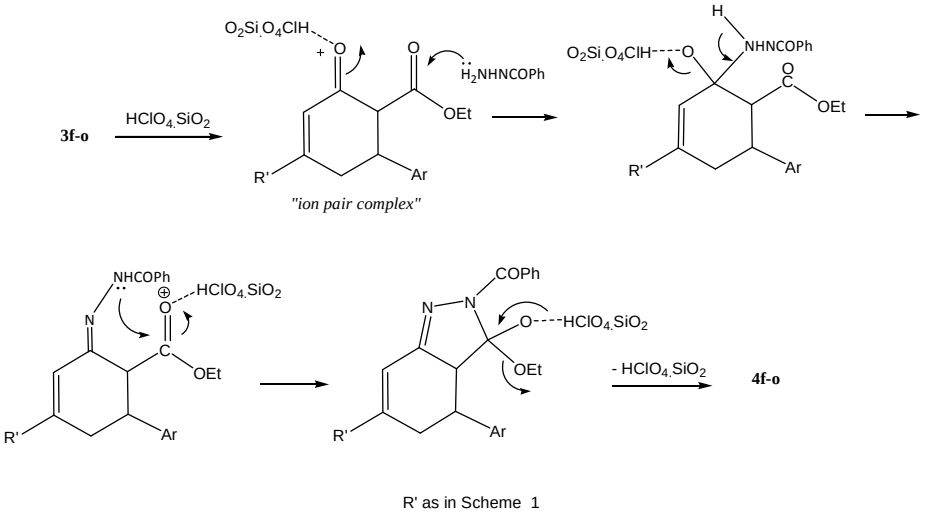
The title compounds were further confirmed by IR, ^{1}H NMR, Mass spectral and elemental analyses. The IR spectral analyses of compound **4f** indicated mainly two medium absorption bands around 1650-1658 cm^{-1}, 1680-1692 cm^{-1} due to the presence of amide carbonyl exocyclic to 5 membered ring of indazolone. Another strong stretching band around 1740-1755 cm^{-1} was observed due to the presence of sydnone carbonyl group.

In case of ^{1}H NMR spectral analysis of compound **4f** showed a set of double doublets around 2.25 to 2.38 ppm due to methylene protons of indazolone ring which couple with the adjacent diastereotopic proton. Another doublet was observed around 2.7 ppm. due to a single proton present on the bridged carbon of indazolone ring. Diastereotopic proton was observed as multiplet due to coupling with adjacent methylene protons and the proton on the bridged carbon atom. Vinylic proton was observed around 6.5 ppm. as singlet. The remaining protons on the substituents present on the phenyl rings appear at their respective regions. Mass spectral analyses of the title compounds showed the molecular ion peaks at their respective molecular masses.



a; R = phenyl, **b**; R = *p*-tolyl, **c**; R = *p*-anisyl, **d**; R = *p*-chlorophenyl, **e**; R = *p*-nitrophenyl, **f**; R = phenyl, Ar = phenyl, **g**; R = *p*-tolyl, Ar = phenyl, **h**; R = *p*-anisyl, Ar = phenyl, **i**; R = *p*-chlorophenyl, Ar = phenyl, **j**; R = *p*-nitrophenyl, Ar = phenyl, **k**; R = phenyl, Ar = *p*-chlorophenyl, **l**; R = *p*-tolyl, Ar = *p*-chlorophenyl, **m**; R = *p*-anisyl, Ar = *p*-chlorophenyl, **n**; R = *p*-chlorophenyl, Ar = *p*-chlorophenyl, **o**; R = *p*-nitrophenyl, Ar = *p*-chlorophenyl.

Scheme 1. Protocol for the formation of compounds 4f-o



R' as in Scheme 1

Scheme 2. Proposed Mechanism of formation of title compounds 4f-o in presence of silica perchloric acid

The mechanism involves the formation of "ion pair complex" due to interaction of silica perchloric acid with the lone pair of electrons on the oxygen atom of carbonyl group of cyclohexene ring of **3f-o** which thus influences the nucleophilic attack of benzhydrazide followed by another attack on the carbonyl group of carbethoxy moiety which results in the formation of five membered ring thus forming the indazolone derivative **4f-o**.

Antimicrobial evaluation

Table 2. MIC values (µg/ml) of the title compounds

Entry No	Minimum inhibitory concentration (MIC) values (µg/ml)			
	S. typhi	***B. pyogenes***	***C. albicans***	***A. niger***
4f	3.00	2.50	3.30	4.00
4g	1.75	1.50	3.30	3.50
4h	3.70	3.40	2.60	3.00
4i	**0.75**	**1.20**	3.10	3.30
4j	1.90	1.60	3.00	2.80
4k	1.30	3.00	4.00	3.40
4l	**0.65**	**0.70**	2.30	4.10
4m	2.70	1.90	3.10	3.30
4n	0.90	1.25	2.00	2.00
4o	**1.00**	3.00	3.00	3.00
Gentamycin	0.50	1.00	-	-
Amphotericin	-	-	2.00	2.00

The synthesized compounds were subjected for antimicrobial evaluation against bacterial strains *S. typhi*, *B. pyogenes* and fungal strains *A. Niger* and *C. albicans.*

Antibacterial activity results

MIC values for the *in vitro* antibacterial studies of the compounds (**4f-o**) and the standard are represented in **Table 2** which range from 0.65-3.70 µg/ml. Antibacterial activity results as compared to the Gentamycin indicated that the compounds **4i**, **4l**, and **4n** have shown excellent activity against both the strains. However, the compound **4o** has shown potent activity only against *S. typhi.*

Antifungal activity results

In case of antifungal activity no compound has shown considerable activity against any of the strains. But it is interesting to note that the compound **4n** with two chorine substituents showed activity similar to the standard drug Amphotericin against both the fungal strains (**Table 2**).

Experimental

Melting points were determined in open capillaries. The IR spectra were recorded on Nicolet Impact 5200 USA FT IR using KBr pellets. ^{1}H NMR spectra were recorded on Bruker 300- MHz FT NMR spectrometer with TMS as internal standard. EI mass spectral analyses were recorded on Shimadzu Japan QP2010 S model spectrometer and elemental analyses were carried out using Heraus CHN rapid analyzer. Silica supported perchloric acid was prepared according to the reported method [13]. The purity of the compounds was checked by thin layer

chromatography (TLC) on silica gel plate using benzene and ethyl acetate. The pharmacological evaluation was carried out at the Biogenics Ltd, Hubli, Karnataka, India.

General procedure for preparation of 2-benzoyl-6-(3-phenyl-sydnon-4-yl)-4-phenyl-2,3a,4,5-tetrahydro-3*H*-indazol-3-one 4f :
Ethyl-2-oxo-6-phenyl-4-(3-phenyl-sydnon-4-yl)cyclohex-3-enecarboxylate **3f** (0.01 mole) was refluxed on water-bath with benzhydrazide in ethanol in presence of silica perchloric acid (10 mol %). After completion of the reaction, the catalyst was recovered by filtration and filtrate was evaporated to dryness to get yellow coloured mass of the compound **4f** which was further crystallised using absolute alcohol to get pale yellow coloured crystals (85 %).

^{1}H NMR ($CDCl_3$ 300 MHz, δ ppm) 6.94-7.78 (15H, m, ArH), 6.73 (1H, q, CH=C), 6.58 (1H, d), 4.40 (1H, m), 4.15 (1H, d), 3.90 (1H, m); IR (KBr) (cm^{-1}): 1752 (sydnone CO), 1688 (N-C=O), 2885, 1652 (C=C); MS (m/z, 70 eV): 478, 315, 178, 119, 91, 76; Anal. Calcd for $C_{28}H_{20}N_4O_4$: C 70.58, H 4.23, N 11.76 found C 70.57, H 4.25, N 11.77.

Similarly, the other compounds **4g-o** were prepared according the above procedure.
2-Benzoyl-6-(3-*p*-tolylsydnon-4-yl)-4-phenyl-2,3a,4,5-tetrahydro-3*H*-indazol-3-one (**4g**) Pale yellow crystals, Yield (80%), MP. 203-05 °C, ^{1}H NMR ($CDCl_3$, 300,MHz, δ ppm): 6.92-7.78 (14H, m, ArH), 6.70 (1H, q CH=C), 6.58 (1H, d) 4.40 (1H, m), 4.15 (1H, d), 3.95 (1H, m), 1.82 (3H, s, CH_3); IR (KBr): 1755 (sydnone CO), 1680 (C=O), 2883, 1655 (C=C); MS (m/z, 70 eV): 492, 315, 178, 119, 93, 76; Anal. Calcd $C_{29}H_{22}N_4O_4$ C 71.01, H 4.52, N 11.42 found C 71.03, H 4.51, N 11.41.

2-Benzoyl-6-(3-*p*-anisylsydnon-4-yl)-4-phenyl-2,3a,4,5-tetrahydro-3*H*-indazol-3-one (**4h)** Pale yellow crystals, Yield (84%), MP. 151-3 °C, ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.90-7.79 (14H, m, ArH), 6.68 (1H, q, CH=C), 6.55 (1H, d), 4.40 (1H, m), 4.15 (1H, d), 3.95 (1H, m), 2.75 (3H, s, OCH_3); IR (KBr): 1753 (sydnone CO), 1680 (C=O), 2883, 1650 (C=C); MS (m/z, 70 eV): 508, 315, 178, 108, 91,76; Anal. Calcd. $C_{29}H_{22}N_4O_5$ C 68.77, H 4.38, N 11.06 found C 68.76, H 4.39, N 11.05.

2-Benzoyl-6-(3-*p*-chlorophenylsydnon-4-yl)-4-phenyl-2,3a,4,5-tetrahydro-3*H*-indazol-3-one (**4i**) ^{1}H NMR ($CDCl_3$ 300 MHz, δ ppm.): 6.80-7.79 (14H, m ArH), 6.70 (1H, q, CH=C), 6.53 (1H, d), 4.40 (1H, m), 4.18 (1H, d), 3.95 (1H, m); IR (KBr) 1750 (sydnone CO), 1680 (C=O), 2883, 1653 (C=C); MS (m/z, 70 eV): 514, 512, 315, 178, 112, 91, 76; Anal. Calcd. $C_{28}H_{19}ClN_4O_4$ C 65.82, H 3.75, N 10.97 found C 65.83, H 3.74, N 10.96.

2-Benzoyl-6-(3-*p*-nitrophenylsydnon-4-yl)-4-phenyl-2,3a,4,5-tetrahydro-3*H*-indazol-3-one (**4j**) ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.80-8.19 (14H, m, ArH) 6.70 (1H, q, CH=C), 6.53 (1H, d), 4.40 (1H, m), 4.18 (1H, d), 3.95 (1H, m); IR (KBr): 1752 (sydnone CO), 1680 (C=O), 2875, 1655 (C=C); MS (m/z, 70 eV): 523, 315,178, 123, 91, 76; Anal. Calcd $C_{28}H_{19}N_5O_6$ C 64.49, H 3.67, N 13.43 found C 64.50, H 3.68, N 13.44.

2-Benzoyl-6-(3-phenylsydnon-4-yl)-4-*p*-chlorophenyl-2,3a,4,5-tetrahydro-3*H*-indazol-3-one (**4k**) ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.80-7.79 (14H, m, ArH), 6.67 (1H, q, CH=C), 6.53 (1H, d), 4.40 (1H, m), 4.18 (1H, d), 3.95 (1H, m); IR (KBr): 1755 (Sydnone CO), 1679 (C=O), 2878, 1655 (C=C); MS (m/z, 70 eV): 514, 512, 349, 176, 112, 91, 76; Anal. Calcd $C_{28}H_{19}ClN_4O_4$ C 65.82, H 3.75, N 10.97 found C 65.80, H 3.74 N 10.98.

2-Benzoyl-6-(3-*p*-tolylsydnon-4-yl)-4-*p*-chlorophenyl-2,3a,4,5-tetrahydro-3*H*-indazol-3-one (**4l**) ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.65-7.79 (13H, m, ArH), 6.65 (1H, q, CH=C), 6.53 (1H, d), 4.40 (1H, m), 4.18 (1H, d), 3.95 (1H, m), 1.78 (3H, s, CH_3); IR (KBr): 1752 (Sydnone CO), 1679 (C=O), 2881, 1650 (C=C); MS (m/z, 70 eV): 528, 526, 349, 176, 112, 92, 76; Anal. Calcd $C_{29}H_{21}ClN_4O_4$ C 66.35, H 4.03, N 10.67 found C 66.37, H 4.04, N 10.68.

2-Benzoyl-6-(3-*p*-anisylsydnon-4-yl)-4-*p*-chlorophenyl-2,3a,4,5-tetrahydro-3*H*-indazol-3-one (**4m**) ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.65-7.79 (13H, m, ArH), 6.73 (1H, q, CH=C), 6.53 (1H, d), 4.40 (1H, m), 4.18 (1H, d), 3.95 (1H, m), 2.74 (3H, s, OCH_3); IR (KBr): 1755 (sydnone CO), 1683 (C=O), 2881, 1655 (C=C); MS (m/z, 70 eV): 544, 542, 349, 176, 108, 92, 76; Anal. Calcd. $C_{29}H_{21}ClN_4O_5$ C 64.39, H 3.91, N 10.36 found C64.37, H 3.92, N 10.35.

2-Benzoyl-6-(3-*p*-chlorophenylsydnon-4-yl)-4-*p*-chlorophenyl-2,3a,4,5-tetrahydro-3*H*-indazol-3-one (**4n**) ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.61-7.77 (13H, m, ArH), 6.74 (1H, q, CH=C), 6.53 (1H, d), 4.40 (1H, m), 4.18 (1H, d), 3.92 (1H, m), IR (KBr): 1751 (sydnone CO), 1681 (C=O), 2878,1655 (C=C); MS (m/z, 70 eV): 549, 547, 349,176,112, 92, 76; Anal. Calcd $C_{28}H_{18}Cl_2N_4O_4$ C 61.66, H 3.33, N 10.27 found C 61.67, H 3.32, N 10.28.

2-Benzoyl-6-(3-*p*-nitrophenylsydnon-4-yl)-4-*p*-chlorophenyl-2,3a,4,5-tetrahydro-3*H*-indazol-3-one (**4o**) ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.61-8.23 (13H, m, ArH), 6.70 (1H, q, CH=C), 6.51 (1H, d), 4.38 (1H, m), 4.16 (1H, d), 3.80 (1H, m); IR (KBr): 1755 (sydnone CO), 1681 (C=O), 2888, 1655 (C=C); MS (m/z, 70 eV): 559, 557, 349, 176, 123, 92, 76; Anal. Calcd $C_{28}H_{18}N_5O_6$ C 60.49, H 3.26, N 12.60 found C 60.50, H 3.27, N 12.61.

Biological assays

Antimicrobial assay

Preliminary screening was conducted for all the compounds at 100 µg/ml concentration against two Gram-positive bacteria, *Salmonella typhi*, *Bacillus pyogenes* and against two fungal strains *Candida albicans* 10145 and *Aspergillus niger* [19-20]. The protocol for the antimicrobial activity assay was as follows:

Dimethylformamide was used as the solvent control. The bacterial cultures were inoculated on Mueller Hinton Agar (Merck) and fungal cultures on Potato Dextrose Agar. Media (20 ml) were poured into each sterilized Petri dish (99 mm) and media were inoculated homogeneously with the liquid cultures by the spread plate method. All the compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain a concentration of 100 µg. Each sample (100 µl) was directly loaded into the wells of agar plates. The plates inoculated with bacteria were incubated at 37 °C for 24 h and the fungal cultures were incubated at 25 °C for 72 h. All the determinations were performed in triplicate. The standards gentamycin (100 µg/ml) for the anti bacterial and amphotericin (100 µg/ml) for anti fungal assays were used as the positive controls and 100 µl of DMSO was used as the negative control. The zones of inhibition were recorded in mm. Different series of dilutions of the compounds were made (0.5, 1.0, … 10.0 µg/ml) to determine the minimum inhibitory concentration (*MIC*).

CONCLUSION

In conclusion, a simple and high yielding method for the preparation of indazole derivatives appended to C_4 carbon of sydnone (**4f-o**) were prepared using silica perchloric acid as heterogenous catalyst. MIC values of the final compounds were evaluated and promising results were obtained for some of the compounds against bacterial and fungal strains.

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