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Synthesis and Antiviral Investigation of New Polynuclear Heterocyclic Compounds Containing Tetrahydroindazole Derivatives.

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ABSTRACT

The antiviral materials or compounds required for the treatment of viruses cause some infectious diseases such as Coxsackievirus B4 (CVB4), rotavirus Wa strain, and adenovirus type 7 are indispensable and of great necessity. The aim of the present study was to synthesize a new series of substituted indazole derivatives obtained from 2-(4-substituted- benzylidene)-4-phenylcyclohexanones and 2,6-bis (4-substituted-benzylidene) -4-phenylcyclohexanone derivatives. The products formed were reacted with 4-hydrazinylbenzoic acid or 2-hydrazino-6-methylbenzothiazole in the presence of cuprous oxide and Cs_2CO_3 as catalysts to give rise to a variety of indazole derivatives in a simple experimental procedure in good yields and short reaction time. The new compounds were fully characterized by spectroscopic and analytical methods. The synthesized compounds were evaluated for their antiviral activity against Coxsackievirus B4, adenovirus type 7 and rotavirus Wa strain. The bioassay results showed that the synthesized compounds possessed variable antiviral bioactivity. Compound (3-Fluoro-7-(fluoromethylene)-4,5,6,7-tetrahydro-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazole (24) exhibited moderate activity against both Coxsackievirus B4 and rotavius Wa strain and potentially promising activity against adenovirus type 7. On the other hand, 3-Chloro-7-(chloromethylene)-4,5,6,7-tetrahydro-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazole (25) and 7-(2,6-Dimethoxybenzylidene)-4,5,6,7-tetrahydro-3-(2,6-dimethoxyphenyl)-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazole (26) revealed potential promising activity against adenovirus type 7, while compounds 25 and 26 revealed promising activities against rotavirus Wa strain.

Keywords: Indazole derivatives, Benzoic acid derivatives, Thiazoloindazole derivatives, Antiviral activity, Coxsackievirus B4, Rotavirus Wa strain, Adenovirus type 7.

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INTRODUCTION

Modern drug discovery recently focused on the discovery of new pharmacological active molecules as antiviral agents. Coxsackievirus B4 (CVB4) is one of six serotypes of the coxsackievirus B group and can cause a broad range of inflammatory diseases, such as pancreatitis, hepatitis, gastroenteritis, and even death in neonates [1]. Furthermore, rotaviruses have very specific cell tropism, infecting enterocytes on the tip of intestinal villi [2], which suggests that specific host receptors must exist. In vitro, they display restricted tropism, binding to a variety of cell lines, but efficiently infecting only those of renal or intestinal epithelium origin [3].

The 51 recognized serotypes of human adenoviruses have been placed in 7 human adenovirus species [4]. However, these viruses may infect respiratory and genitourinary sites and have been recognized for decades as the primary causes for acute respiratory disease (ARD), gastrointestinal infection, and fever [5].

Previous antiviral studies have primarily focused on the development of drugs that are nucleoside analogues but recently, non-nucleoside derivatives [6-8] were also considered and recognized, among those are some novel indazole derivatives that were reported to exhibit high antiviral activities against hepatitis A virus, hepatitis C virus (HCV) and Herpex simplex virus (HSV)[9,10] respectively.

In addition, tetrahydroindazoles and their substituents have attracted attention by virtue of their sunique pharmacological applications [11]. Moreover, Indazole derivatives are known to possess a wide range of biological activities including nitric oxide (NO) synthase inhibition [12], analgesic [13], anti-inflammatory [14] and antiviral activities [15]. They also possess HIV protease inhibitor activity [16], anti-fertility [17], anticancer [18], and HCV [19] as well as potential anti-parasitic effects [20]. As a result of those findings, they were used in many pharmaceutical applications [21,22].

Indazole related drugs such as APINACA (AKB48) and AKB48-F possess high therapeutic properties and have recently attracted the attention of pharmaceutical chemists to synthesize novel chemotherapeutic agents as antineoplastic agents [23]. Those agents were synthesized using heterogeneous catalysts such as cuprous oxide and Cs_2CO_3 in organic synthesis as reported [24,25].

Motivated by those promising findings and our interest in the synthesis of new heterocyclic derivatives, and after screening them biologically in our laboratory [26-28], we hereby describe a facile synthesis of different new heterocyclic compounds of indazole derivatives with the aim of exploring their antiviral activity against Coxsackievirus B4, adenovirus type 7 and rotavirus Wa strain using the plaque assay method.

RESULTS AND DISCUSSION

The reaction sequences employed for the synthesis of the new target indazole derivatives are illustrated in Schemes 1,2 and 3.

The condensation reaction of (one or two mole) of 4-phenycyclohexanone **1** with one mole or two various aldehydes $\mathbf{2}_{a-d}$ namely: 4-florobenzaldehyde, 4-chlorobenzaldehyde,4-(N,N dimethylamino)benzaldehyde, 2,6 dimethoxybenzaldhyde or in the presence of cuprous oxide and Cs_2CO_3 as catalysts afforded the corresponding benzylidene-4-phenylcyclohexanone derivatives namely2-(4-fluorobenzylidene)-4 phenylcyclohexanone, 2-(4-chloro-benzylidene)-4-phenylcyclo-hexanone, 2-(2,6-dimethoxybenzylidene)-4-phenyl-cyclohexanone, or 2-(4-(dimethyl-amino)benzylidene)-4-phenylcyclohexanone,respectively (**3-6**). The structures of compounds **3-6** were confirmed by spectral (IR, ^{1}H NMR, mass spectra) as well as elemental analysis.

The IR spectrum of compound **1** *as* an example showed absorption bands in the regions of 1177 $cm^{-1}$for C-F, 1679 cm^{-1} for C=O, in addition to MS at m/z 280. ^{1}H- NMR (500 MHz, DMSO-d6, δ ppm) showed a signal at 5.48 (1H, s, CH) corresponding to the methane proton.

The reaction of compounds **3-6** with 4-hydrazinylbenzoic acid $\mathbf{11}_{a,b}$ in acidic medium with the presence of silica sulfuric acid, led to the formation of the corresponding 4,5,6,7-tetrahydro-5-phenylindazol-

2-yl)benzoic acid derivatives and were obtained in good yield and short reaction time, namely 7-(4-fluorobenzylidene)-3-(4-fluorophenyl)-4,5,6,7-tetrahydro-5-phenylindazol-2-yl)benzoic acid **(12)**, 7-(4-chlorobenzylidene)-3-(4-chlorophenyl)-4,5,6,7-tetrahydro-5-phenylindazol-2-yl)benzoic acid **(13)**, 7-(2,6-dimethoxybenzylidene)-4,5,6,7-tetrahydro-3-(2,6-dimethoxyphenyl)-5-phenylindazol-2-yl)benzoic acid **(14)** or 7-(4-(dimethylamino)benzylidene)-3-(4-(dimethylamino)phenyl)-4,5,6,7-tetrahydro-5-phenylindazol-2-yl) benzoic acid **(15)**, respectively.
Acetic acid and acetic anhydride were used to provide the acidic medium needed for the reaction medium, which played an important role in cyclization and *in situ* cleavage of the hydrazine reagents to produce (1*H*) indazoles as reported [29,30]

The IR spectra of the indazoles **12-19** displayed strong absorption bands between 1695-1725cm^{-1}indicating the presence of CO carboxylic acid groups. In addition to absorption bands at 1640-1650 cm^{-1} for C=N groups and 2120-2140 $cm^{-1}$for N-N. The indazole derivatives **12-19** were fully characterized by spectral data as well as elemental analysis *(see Experimental)*

In addition, when two moles of aldehydes were reacted with 4-phenylcyclohexanone in the presence of cuprous oxide and Cs_2CO_3 as catalysts in dimethylformamide-dimethylacetal DMF-DMA, they afforded compounds **7-10** which when reacted with one mole of4-hydrazinylbenzoic acid in acidic medium and in the presence of silica sulfuric acid as a catalyst formed the corresponding tetrahydro-(5-phenylindazol-2-yl)benzoic acid derivatives **20-27**. The heterogeneous catalysts are recovered and recycled several times without loss of its activity. The structures of compounds **20-23** and **24-27** are well supported by their spectral data and elemental analysis. The IR spectra of **20-23** showed absorption at 1638- 1648 cm^{-1} for C=N *(see experimental*)

MATERIALS AND METHODS

General methods

All the chemical reagents used for the synthesis were obtained from Merck, Aldrich and Fluka. All melting points were determined using Electro thermal Capillary melting point apparatus (Stuart, SMP30, UK) were uncorrected. Microanalyses, of the compounds were performed in the Micro analytical Laboratories, Cairo University, Egypt. The IR spectra (4000- 400 cm^{-1}) were recorded using KBr pellets in a Jasco FT/IR 300E Fourier transform infrared spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded using Joel EX-270 MHz NMR spectrophotometers, National Research Centre. Chemical shifts are reported in parts per million (ppm) from the tetramethylsilane resonance in the indicated solvent. The mass spectra were carried out using GC-MS Finnegan spectrometer at 70ev (Thermo. Inst. Sys. Inc.,USA), National Research Center, Cairo, Egypt. Monitoring of the progress of all reactions and homogeneity of the synthesized compounds were carried out using TLC (silica gel pre-coated aluminum cards with fluorescent indicator at 254 nm (Merck, Germany). Visualization was performed by illumination with a UV light source. 2-hydrazino-6-methylbenzothiazole was prepared according to the reported procedure [31]**.**

Synthesis

Synthesis of benzylidene-4-phenylcyclohexanone derivatives 3-6, General Procedure:

Equimolar quantities of 4-phenylcyclohexanone (0.01 mmol) and substituted aldehydes namely:4-florobenxaldehyde, 4-chlorobenzaldehyde, 2,6 dimethoxybenzaldhyde or 4-(N,N dimethylamino) benzaldehyde (0.01 mmol); was dissolved in dioxane (10 ml) and DMF-DMA (0.01 mmol) , then cuprous oxide (5mol %)and Cs_2CO_3 (2.0 equiv.) was added and stirred vigorously for 2-3h at 120 °C .Completion of the reaction was monitored by TLC. The reaction mixture was then centrifuged to separate cuprous oxide and was extracted with ethyl acetate. The solvent was evaporated under reduced pressure and the precipitate obtained was washed with water and recrystallized from acetone to form compounds 3-6.

2-(4-Fluorobenzylidene)-4-phenylcyclohexanone (3)

Yield 76 %, mp 170-172 ºC ; IR: cm^{-1} 3170 (CH-aromatic) ,1679 (C=O), 1589 (C=C), 1177(C-F); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 4.20-4.35 (6H, m, $3CH_2$), 5.48 (1H, m, CH) , 6.70-6.85 (5H, m, Ar-H , the sp^2methine proton), 6.86-7.00 (5H, m, Ar-H), Anal.Calc. For $C_{19}H_{17}FO$ (280. 336): C (81.40%), H (6.11%), F (6.78%). Found: C (81.44%), H (6.14%), F (6.72%); M: m/z (%): 280 (M^+) (39.80)

2-(4-chlorobenzylidene)-4-phenylcyclohexanone (4)

Yield 74 %, mp 148-150 ºC; IR: cm^{-1} 3179 (CH-aromatic), 1690 (C=O), 1580 (C=C), 1096(C-Cl); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 4.28-4.39 (6H, m, $3CH_2$), 5.37 (1H, m, CH) , 6.75-6.80 (5H, m, Ar-H), 6.90-7.00 (4H, m, Ar-H), 7.15 (1H,s, the sp^2methine proton); Anal.Calc. For $C_{19}H_{17}ClO$ (296.791): C (76.89%), H (5.77%), Cl (11.95%). Found: C (76.83%), H (5.82%), Cl (11.98%); MS m/z (%): 298 ($M^+ + 2$) (38.60).

2-(2,6-Dimethoxybenzylidene)-4-phenylcyclohexanone (5)

Yield 76 %, mp 190-192 ºC; IR: cm^{-1} 3169 (CH-aromatic), 1689 (C=O), 1588 (C=C); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 3.75 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.20-4.30 (6H, m, $3CH_2$), 5.20 (1H, m, CH) , 6.75-6.80 (3H, m, Ar-H), 6.90-7.00 (5H, m, Ar-H), 8.25 (1H,s, the sp^2methine proton); Anal.Calc. For $C_{21}H_{22}O_3$ (322.398): C (78.23%), H (6.88%), N (6.56%). Found: C (78.27%), H (6.82%), N (6.60%); MS at m/z (%): 322 (M^+) (40.90).

2-(4-(Dimethylamino)benzylidene)-4-phenylcyclohexanone (6)

Yield 79 %, mp 150-152 ºC ; IR: cm^{-1} 3177 (CH-aromatic), 1680 (C=O),1582 (C=C););^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.95 (6H, s, N(CH3)2, 4.18-4.26 (6H, m, $3CH_2$), 5.47 (1H, m, CH) , 6.75-6.80 (5H, m, CH aromatic), 6.90-7.00 (4H, m, CH aromatic), 8.25 (1H,s, the sp^2methine proton); Anal.Calc. For $C_{21}H_{22}O_3$ (305.413): C (82.58%), H (7.59%), N (4.59%). Found: C (82.55%), H (7.53%), N (4.54%); MS at m/z (%): 305 (M^+) (44.60).

Synthesis of benzylidene-4-phenylcyclohexanone derivatives 7-10, General procedure:

A mixture of 4-phenylcyclohexanone (0.1 mmol) and substituted aldehydes namely: 4-florobenzaldehyde, 4-chlorobenzaldehyde, 2,6 dimethoxybenzaldhyde, or 4-(N,N dimethylamino)benzaldehyde (0.2 mmol); was dissolved in dimethylcarbonate (10 ml), then cuprous oxide(5.0 mol %) and Cs_2CO_3 (2.0 equiv.) were added and stirred vigorously for 3-4h at 100 °C, until completion of the reaction monitored by TLC. The reaction mixture was then centrifuged to separate cuprous oxide and then extracted with ethyl acetate (3 x 20 ml). The excess of solvent was removed under vacuum and the solid residue obtained was washed with water, filtered and recrystallized from absolute ethanol to produce compounds **7-10**.

2,6-Bis(4-fluorobenzylidene)-4-phenylcyclohexanone (7)

Yield 70%, mp 158-160 ºC; IR: cm^{-1} 3168-3179 (CH-aromatic), 1686 (C=O); 1597(C=C), 1170 (C-F); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.25-2.35 (4H, m, $2CH_2$), 5.30 (1H, m, CH) , 6.90-7.00 (5H, m, CH- aromatic), 7.15-7.30 (8H, m, CH aromatic), 7.70 (2H, s, 2CH sp^2methine protons); Anal.Calc. For $C_{26}H_{20}F_2O$ (386.433): C (80.81%), H (5.22%), N(9.83%). Found: C (80.81%), H (5.22%), F (9.83%); MS m/z (%): 386 (M^+) (38.90).

2,6-Bis(4-chlorobenzylidene)-4-phenylcyclohexanone (8)

Yield 74 %, mp 169-171 ºC; IR: cm^{-1} 3160-3170 (CH-aromatic), 1688 (C=O), 1587(C=C), 1097 (C-Cl); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.38-2.45 (4H, m, $2CH_2$), 5.40 (1H, m, CH) , 6.90-7.00 (5H,m, CH aromatic), 7.15-7.30 (8H, m, CH aromatic), 7.60 (2H, s, 2CH sp^2methine protons);Anal.Calc. For $C_{26}H_{20}Cl_2O$ (419.342): C (74.47%), H (4.81%), N (6.83%), Cl (16.91%). Found: C (74.47%), H (4.81%), N (6.88%), Cl (16.91%).

2,6-Bis(2,6-dimethoxybenzylidene)-4-phenylcyclohexanone (9)

Yield 78 %, mp 150-152 ºC; IR: cm^{-1} 3165-3178 (CH-aromatic), 1690 (C=O), 1585(C=C) ; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.18-2.30 (6H, m, $3CH_2$), 3.70 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 5.35 (1H, m, CH) , 6.75-6.80 (5H, m, CH aromatic), 7.15-7.30 (6H, m, CH aromatic, 7.76 (2H, s, 2CH sp^2methine protons); Anal.Calc. For $C_{30}H_{30}O_5$ (470.556): C(76.57%), H (6.43%). Found: C (76.57%), H (6.43%), N (9.30). Found: C (76.52%), H (6.48%), N (9.33); MS at m/z (%): 470 (M^+) (34.80).

2,6-Bis(4-(dimethylamino) benzylidene)-4-phenylcyclohexanone (10)

Yield 75 %, mp 138-140 ºC; IR: cm^{-1} 3177-3185 (CH-aromatic), 1685 (C=O), 1595 (C=C); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.19-2.29 (4H, m, $2CH_2$), 3.70 (6H, s, $N(CH3)_2$), 5.45 (1H, m, CH) , 6.75-6.80 (5H, m, CH aromatic), 7.15-7.30 (8H, m, CH aromatic), 7.70 (2H, s, 2CH sp^2 methine protons); Anal.Calc. For $C_{30}H_{32}N_2O$ (436.588): C (82.53%), H (7.39%), N(6.42%). Found: C (82.53%), H (7.39%), N (6.42%); MS m/z (%): 435 (M^+-1) (32.90).

Synthesis of tetrahydro-(5-phenylindazol-2-yl) benzoic acid derivatives 12-15, General procedure:

A mixture of compounds **3-6**(0.01 mmol) and 4-hydrazinylbenzoic acid **11_a** (0.01mmol) in the presence of acetic acid (5ml) and acetic anhydride (1ml) mixture and in the presence of silica sulfuric acid (5 mol%) were heated under reflux with stirring for 3-4h. The reaction progress was monitored using TLC (hexane: ETOAC, 8:2). After completion, the reaction mixture was poured into crushed ice. The precipitate that formed was filtered, washed with cold water several times and dried under vacuum. The product was purified by recrystallization from absolute ethanol to form compounds **12-15** in good yields.

4-(3-(4-Fluorophenyl)-4,5,6,7-tetrahydro-5-phenylindazol-2-yl)benzoic acid (12)

Yield 82 %, mp 166-168 ºC; IR: cm^{-1} 3345 (OH), 3178 (CH-aromatic), 1645 (C=N), 1175 (C-F); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 4.25-4.35(6H, m, $3CH_2$), 5.40 (1H, m, CH), 6.75-6.80 (4H, m, Ar-H), 6.90-7.00 (5H, m, Ar-H), 7.15-7.30 (4H, m, CH aromatic), 11.20 (1H, s, OH); Anal.Calc. For $C_{26}H_{21}FN_2O_2$ (412.456): C (75.71%), H (5.13%), N (6.79%), F (4.61%). Found: C (75.76%), H (5.18%), N (6.74%), F (4.68%).

4-(3-(4-Chlorophenyl)-4,5,6,7-tetrahydro-5-phenylindazol-2-yl)benzoic acid (13)

Yield 80 %, mp 174-176 ºC ; IR: cm^{-1} 3340 (OH), 3179 (CH-aromatic), 1650 (C=N), (C-Cl); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 4.28-4.39(6H, m, $3CH_2$),5.37 (1H, m, CH) , 6.75-6.80 (5H, m, Ar-H), 6.90-7.00 (4H, m, Ar-H), 7.15-7.30 (4H, m, Ar-H), 11.30 (1H, s, OH); Anal.Calc. For $C_{26}H_{21}ClN_2O_2$ (428.910): C (72.81%), H (4.94%), N (6.53%), Cl (8.27%). Found: C (72.88%), H (4.90%), N (6.59%), Cl (8.32%).

4-(4,5,6,7-Tetrahydro-3-(2,6-dimethoxyphenyl)-5-phenylindazol-2-yl)benzoic acid (14)

Yield 84 %, mp 186-188 ºC ; IR: cm^{-1} 3348 (OH), 3179 (CH-aromatic), 1640 (C=N); ^{1}H- NMR (500 MHz, DMSO-d6, δ ppm), 3.70 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.20-4.30 (6H, m, $3CH_2$), 5.20 (1H, m, CH) , 6.75-6.80 (5H, m, Ar-H), 6.90-7.00 (4H, m, Ar-H), 7.15-7.30 (3H, m, CH-aromatic), 11.25 (1H, s, OH); Anal.Calc. For $C_{28}H_{26}N_2O_4$ (428.910): C (73.99%), H (5.77%), N (6.16%). Found: C (73.92%), H (5.72%), N (6.13%); MS at m/z (%):429 (M^++1) (42.90).

4-(3-(4-(Dimethylamino)phenyl)-4,5,6,7-tetrahydro-5-phenylindazol-2-yl)benzoic acid (15)

Yield 82 %, mp 190-192 ºC; IR: cm^{-1} 3349 (OH), 3177 (CH-aromatic), 1645 (C=N); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.95 (6H, s, N (CH3)2) , 4.18-4.26 (6H, m, $3CH_2$), 5.47 (1H, m, CH) , 6.75-6.80 (5H, m, CH aromatic), 6.90-7.00 (4H, m, CH-aromatic), 7.15-7.30 (4H, m, CH-aromatic), 11.20 (1H, s, OH) ; Anal.Calc. For $C_{28}H_{27}N_3O_2$ (437.533): C (76.86%), H (6.22%), N (9.60%). Found: C (76.81%), H (6.22%), N (9.65%); MS at m/z (%): 437 (M^+) (45.90).

Synthesis of tetrahydro-benzodthiazol-2-yl)-5-phenyl-2H-indazole derivatives 16-19, General procedure:

A mixture of (0.1 mmol) of compounds **3-6** and 2-Hydrazino -6- methylbenzothiazole **11_b** (0.1mmol) in acetic acid (5ml) and acetic anhydride (1ml) mixture in the presence of *P*-toluene sulfonic acid *P*TSA (5 mol%) were heated under reflux with stirring for 5-6h. The reaction progress was followed using TLC (hexane: ETOAC, 8:2). After completion of the reaction, the reaction mixture was poured into crushed ice and brine (20ml). The precipitate that formed was filtered, washed with cold water and dried under vacuum. The product was recrystallized from absolute ethanol to afford compounds **16-19** in good yields.

3-Fluoro-4,5,6,7-tetrahydro-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazole (16)

Yield 78 %, mp 215-217 ºC; IR: cm^{-1} 3175 (CH-aromatic), 1645 (C=N), 1569(C=C), 1172 (C-F); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.18 (3H, s, CH_3), 4.20-4.30 (6H, m, $3CH_2$), 5.38 (2H, m, CH), 6.75-6.80(5H, m, CH-aromatic), 6.95-7.15 (4H, m, CH- aromatic), 7.25-7.38 (3H, m, CH- aromatic); Anal.Calc. For $C_{21}H_{18}FN_3S$ (363.451): C (69.40%), H (4.99%), N (11.56%), F (5.23%), S (8.82%). Found: C (69.45%), H (4.93%), N (11.60%), F (5.28%), S (8.87%).

3-Chloro-4,5,6,7-tetrahydro-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazole (17)

Yield 75 %, mp 226-228 ºC; IR: cm^{-1} 3176 (CH-aromatic), 1646 (C=N), 1549 (C=C), 1160 (C-Cl); ^{1}H- NMR (500 MHz, DMSO-d6, δ ppm), 2.25 (3H, s, CH_3), 4.25-4.36 (6H, m, $3CH_2$), 5.48 (2H, m, CH), 6.90-7.00(5H, m, CH- aromatic), 7.10-7.15(4H, m, CH- aromatic), 7.20-7.30 (3H, m, CH- aromatic), Anal.Calc. For $C_{21}H_{18}ClN_3S$ (379.906): C (66.39%), H (4.78%), N (11.06%), Cl (9.33%), S (8.44%). Found: C (66.33%), H (4.83%), N (11.10%), Cl (9.30%), S (8.48%).

4,5,6,7-Tetrahydro-3-(2,6-dimethoxyphenyl)-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazole (18)

Yield 78 %, mp 233-235 ºC; IR: cm^{-1} 3177 (CH-aromatic), 1640 (C=N), 1590(C=C); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.29 (3H, s, CH_3), 4.38-4.48 (6H, m, $3CH_2$), 3.78 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 5.55 (1H, m, CH), 6.75-6.80 (5H, m, CH- aromatic), 6.90-7.20(4H, m, CH- aromatic), 7.25-7.39 (3H, m, CH- aromatic) ; Anal.Calc. For $C_{29}H_{27}N_3O2S$ (481.609): C (72.32%), H (5.65%), N (8.72%), S (6.66%). Found: C (72.28%), H (5.60%), N (8.75%), S (6.61%); MS at m/z (%): 480 (M^+-1) (49.90).

4-(4,5,6,7-Tetrahydro-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazol-3-yl)-N,N-dimethylbenzenamine (19)

Yield 76 %, mp 244-246 ºC; IR: cm^{-1} 3170 (CH-aromatic), 1645 (C=N), 1580 (C=C) ; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.29 (3H, s, CH_3), 2.99 (6H, s, N(CH3)2), 4.28-4.36 (6H, m, $3CH_2$), 5.05 (1H, m, CH) 6.75-6.80(5H, m, CH- aromatic), 6.90-7.00 (4H, m, CH- aromatic), 7.25-7.35 (3H, m, CH- aromatic); Anal.Calc. For $C_{29}H_{28}N_4S$ (464.624): C (74.97%), H (6.07%), N (12.06%), S (6.90%). Found: C (74.92%), H (6.00%), N (12.01%), S (6.95%); MS at m/z (%): 464 (M^+) (60.30).

Synthesis of compounds 7-(4-benzylidene)-tetrahydro-3-(4-phenyl)-5-phenylindazol-2-yl)benzoic acid derivatives 20-23 and/or tetrahydro-2-(6-methylbenzothiazol-2-yl)-5-phenyl-2H-indazole derivatives 24-27,

General procedure:

A mixture of compounds **7-10** (0.01mmol) and 4-hydrazinylbenzoic acid **11_a**(0.01mmol), in presence of acetic acid (5ml) and acetic anhydride (1ml) with silica sulfuric acid (5 mol%). The reaction mixture was heated under reflux for 4-5h. The reaction progress was monitored using TLC (hexane: ETOAC, 8:2). The precipitate formed was filtered, washed with cold water and dried under vacuum. The product was then recrystallized from acetone.

7-(4-Fluorobenzylidene)-3-(4-fluorophenyl)-4,5,6,7-tetrahydro-5-phenylindazol-2-yl)benzoic acid (20)

Yield 82 %, mp 270-272 ºC; IR: cm^{-1} 3340 (OH), 3170 (CH-aromatic), 1648 (C=N), 1128 (C-F);^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.25-2.35 (4H, m, $2CH_2$), 5.30 (1H, m, CH) , 6.90-7.00 (5H, m, CH- aromatic), 7.15-7.30 (8H, m, CH- aromatic), 7.45-7.60 (4H, m, CH- aromatic), 7.70 (1H, s, CH), 11.29 (1H, s, OH); Anal.Calc. For $C_{33}H_{24}FN_2O_2$ (518.553): C (78.43%), H (4.67%), N (5.40%), F (7.33%). Found: C (78.47%), H (4.62%), N (5.45%), F (7.39%); MS m/z (%): 518 (M^+) (45.90).

7-(4-Chlorobenzylidene)-3-(4-chlorophenyl)-4,5,6,7-tetrahydro-5-phenylindazol-2-yl)benzoic acid (21)

Yield 80 %, mp 180-182 ºC; IR: cm^{-1} 3345 (OH), 3170 (CH-aromatic), 1640 (C=N), 865 (C-Cl); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.38-2.45 (4H, m, $2CH_2$), 5.40 (1H, m, CH), 6.90-7.00 (5H, m, CH- aromatic), 7.15-7.30 (8H, m, CH- aromatic), 7.45-7.60 (4H, m, CH- aromatic),7.60 (1H, s, CH), 11.35 (1H, s, OH) ; Anal.Calc. For $C_{33}H_{24}Cl_2N_2O_2$ (551.462): C (71.87%), H (4.39%), N (5.08%), Cl (12.86%). Found: C (71.81%), H (4.32%), N (5.12%), Cl (12.82%).

7-(2,6-Dimethoxybenzylidene)-4,5,6,7-tetrahydro-3-(2,6-dimethoxyphenyl)-5-phenylindazol-2-yl)benzoic acid (22)

Yield 84 %, mp 190-192 º; IR: cm^{-1} 3349 (OH), 3175 (CH-aromatic), 1638 (C=N); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.18-2.30 (4H, m, $2CH_2$), 3.70 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 5.35 (1H, m, CH), 6.75-6.80 (5H, m, CH -aromatic), 6.90-7.00 (6H, m, CH- aromatic), 7.40-7.50 (4H, m, CH- aromatic), 7.76 (1H, s, CH), 11.33 (1H, s, OH); Anal.Calc. For $C_{37}H_{34}N_2O_6$ (602.676): C (73.74%), H (5.69%), N (4.65%). Found: C (73.79%), H (5.62%), N (4.71%). MS at m/z (%): 602 (M^+) (38.90).

7-(4-(Imethylamino)benzylidene)-3-(4-(dimethylamino)phenyl)-4,5,6,7-tetrahydro-5-phenylindazol-2-yl)benzoic acid (23)

Yield 89 %, mp 196-198 ºC; IR: cm^{-1} 3350 (OH), 3170 (CH-aromatic), 1645 (C=N); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.19-2.29 (4H, m, $2CH_2$), 3.70 (6H, s, N $(CH3)_2$), 5.45 (1H, m, CH), 6.75-6.80 (5H, m, C aromatic), 6.90-7.00 (8H, m, CH- aromatic), 7.40-7.50 (4H, m, CH- aromatic), 7.70 (1H, s, CH), 11.28 (1H, s, OH) ; Anal.Calc. For $C_{37}H_{36}N_4O_2$ (569.707): C (78.14%), H (6.38%), N (9.85%). Found: C (78.10%), H (6.44%), N (9.90%); MS at m/z (%): 569 (M^+) (45.80).

3-Fluoro-7-(fluoromethylene)-4,5,6,7-tetrahydro-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazole (24)

Yield 70 %, mp 250-252 ºC; IR: cm^{-1} 3178 (CH-aromatic), 1639 (C=N), 1567(C=C), 1170-1178 (2C-F); ^{1}H- NMR (500 MHz, DMSO-d6, δ ppm), 2.21 (3H, s, CH_3), 4.40-4.50 (4H, m, $2CH_2$), 5.33 (2H, m, CH), 6.75-6.80 (5H, m, CH-aromatic), 6.95-7.15 (8H, m, CH-aromatic), 7.25-7.35 (3H, m, CH-aromatic), 7.58 (1H, s, CH); Anal.Calc. For $C_{22}H_{17}F_2N_3S$ (393.111): C (67.16%), H (4.36%), N (10.68%), F (9.66%), S (8.15%). Found: C (67.21%), H (4.31%), N (10.73%), F (9.60%), S (8.11%).

3-Chloro-7-(chloromethylene)-4,5,6,7-tetrahydro-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazole (25)

Yield 80 %, mp 260-262 ºC; IR: cm^{-1} 3176 (CH-aromatic), 1656 (C=N), 1549 (C=C), 1160-1170 (2C-Cl); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.25 (3H, s, CH_3), 4.24-4.35 (4H, m, $2CH_2$), 5.46 (2H, m, CH), 6.95-7.10 (5H, m, CH- aromatic), 7.15-7.25 (8H, m, CH- aromatic), 7.31-7.45 (3H, m, CH- aromatic), 7.65 (1H, s, CH); Anal.Calc. For $C_{22}H_{17}Cl_2N_3S$ (425.052): C (61.97%), H (4.02%), N (9.86%), Cl (16.63%), S (7.52%). Found: C(61.92%), H (4.05%), N (9.89%), Cl (16.68%),S (7.50%).

7-(2,6-Dimethoxybenzylidene)-4,5,6,7-tetrahydro-3-(2,6-dimethoxyphenyl)-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazole (26)

Yield 80 %, mp 245-247 ºC; IR: cm^{-1} 3175 (CH-aromatic), 1648 (C=N), 1595(C=C); ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.16 (3H, s, CH_3) , 3.73 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 4.37-4.48 (4H, m, $2CH_2$), 5.48 (1H, m, CH), 6.75-6.85 (5H, m, CH- aromatic), 7.01-7.15 (6H, m, CH- aromatic), 7.25-7.35 (3H, m, CH- aromatic), 7.68 (1H, s, CH); Anal.Calc. For $C_{38}H_{35}N_3O_4S$ (629.767): C (72.47%), H (5.60 %), N (6.67%), S (6.09%).Found: C (72.42%), H (5.66 %), N (6.72%), S (6.04%). MS at m/z (%): 430 (M^++1) (58.90).

7-(4-(Dimethylamino)benzylidene)-4,5,6,7-tetrahydro-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazol-3-yl)-N,N-dimethylbenzenamine (27)

Yield 82 %, mp251-253 ºC; IR: cm^{-1} 3172 (CH-aromatic), 1640 (C=N), 1585 (C=C) ; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm), 2.18 (3H, s, CH_3), 2.96 (6H, s, $N(CH3)_2$), 4.25-4.35 (4H, m, $2CH_2$), 6.80-6.90 (5H, m, CH-aromatic), 7.01-7.15 (8H, m, CH-aromatic), 7.20- 7.35(3H, m, CH-aromatic), 7.70 (1H, s, CH); Anal.Calc. For $C_{38}H_{37}N_5S$ (595.277): C (76.60%), H (6.26 %), N (11.75 %), S (5.38 %). Found: C (76.65%), H (6.22 %), N (11.70 %), S (5.43 %); MS m/z (%): 594 (M^+-1) (55.80).

Pharmacology

***In vitro* antiviral activity**

Some of the synthesized compounds were evaluated for their effects on Coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain *in vitro* using the plaque assay technique.

Cytotoxicity assay

Cytotoxicity assay was done according to Simoes method (Simões et al ,1999) [32] and Walum method (Walum et al , 1990) [33].Briefly, all samples (50 mg) were dissolved in 1 ml of DMSO. Decontamination of samples was done by adding 24µL of 100× of antibiotic–antimycotic mixture to 1 ml of each sample. 1:2 (dilutions) were done to 100µL of originally dissolved samples and 100µL of each dilutions were inoculated in Hep-2, BGM and MA104 cell lines (obtained from the Holding Company for Biological Products & Vaccines VACSERA, Egypt) previously cultured in 96 multi well plates (Greiner-Bio one, Germany) to estimate the nontoxic dose of the tested samples. Cytotoxicity assay was done using cell morphology evaluation by inverted light microscope and cell viability test applying trypan blue dye exclusion method.

Cell morphology evaluation by inverted light microscopy

Hep-2, BGM, and MA104 cell cultures (2×10^5 cells/mL) were prepared separately in 96-well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation at 37^0C in a humidified 5% (v/v) CO_2 atmosphere cell monolayers were confluent, the medium was removed from each well and replenished with 100µL of (1:2 dilutions) of different samples tested prepared in DMEM (GIBCO BRL). For cell controls 100µL of DMEM without samples was added. All cultures were incubated at 37^0C in a humidified 5% (v/v) CO_2 atmosphere for 72h. Cell morphology was observed daily for microscopically detectable morphological alterations, such as loss of confluence, cell rounding and shrinking, and cytoplasm granulation and vacuolization. Morphological changes were scored (Simoes et al., 1999)[32].

Cell viability assay

The assay was done by trypan blue dye exclusion method (Walum et al., 1990)[33] Hep-2, BGM, and MA104 cell cultures (2×10^5 cells/mL) were grown in 12-well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation, the same cytotoxicity assay was described above for tested samples and was followed by applying 100µL of tested samples dilutions (1:2 dilutions) per well. After 72 h the medium was removed, cells were trypsinized and an equal volume of 0.4% (w/v). Trypan blue dye aqueous solution was added to cell suspension. Viable cells were counted under the phase contrast microscope.

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Determination of adenovirus type 7, Coxsackievirus B4, rotavirus Wa strain titers using the plaque assay:

Non-toxic dilutions were mixed (100µl) with 100µl of different doses of adenovirus type 7 (1×10^5, 1×10^6, 1×10^7) and the same doses of Coxsackievirus B4, and rotavirus Wa strain. The infectivity of the rotavirus stocks were activated with 10 µg/ml trypsin for 30 min at 37°C. The mixture was incubated for 1/2 h in 37°C. The inoculation of (100µl) 10 fold dilutions of treated and untreated Adenovirus type 7, Coxsackievirus B4, and rotavirus Wa strain was carried out separately into Hep 2, BGM, and MA104 cell lines for adenovirus type 7 Coxsackievirus B4, and rotavirus Wa strain respectively in 12 multi well- plates. After 1 hour of incubation at 37°C in a 5% CO_2-water vapor atmosphere, the plates were rocked intermittently to keep the cells from drying. After adsorption, 1 ml of 2X media (Dulbecco's Modified Eagle Medium, Gibco- BRL (DMEM) plus 1ml 1% agarose was added to each well, 0.5 µg/ml of trypsin was added to the media-agarose mixture in the case of rotavirus Wa strain and the plates were incubated at 37°C in a 5% CO_2-water vapor atmosphere. After the appropriate incubation period, the cells were stained with 0.4% crystal violet after formalin fixation, and the number of plaques counted. The viral titers were then calculated, and expressed as plaque-forming units per milliliter (pfu/ml) (Schmidtke et al, 1998)[34].

Table 1 described the non-toxic doses of the tested compounds on MA104, Hep2, and BGM cell lines. Compounds 19, 24, 25 and 26 revealed potentially promising activities against Coxsackievirus B4 as shown by the results in Table 2. Compound 24 exhibited a moderately potent activity against Coxsackievirus B4 and rotavirus Wa strain as demonstrated in tables 3 and 4 respectively. Compounds 24 and 25 revealed potentially promising activity against adenovirus type 7 as demonstrated in Table 3. Compounds 24, 25 and 26 possess potentially promising antiviral activities against rotavirus Wa strain as shown in Table 4. The activities of the tested compounds were not compared with a reference drug due to the fact that no antiviral agents were used until now against the viruses Coxsackievirus B4, adenovirus type 7 and rotavirus Wa strain.

Table 1. Non-toxic doses of tested compounds on MA104, Hep2, and BGM cell lines.

Tested compounds	Non -toxic dose on MA104 cell line (µg/ml)	Non-toxic dose on Hep2 cell line (µg/ml)	Non -toxic dose on BGM cell line (µg/ml)
1	40	50	50
2	40	40	40
3	30	40	40
4	40	40	40
5	30	30	40
7	40	40	50
8	30	30	30
9	30	30	30
10	30	30	40
11	30	30	30
17	60	60	60
18	40	50	50
19	30	30	40
20	40	40	40
21	30	30	40
23	30	40	40
24	40	50	60
25	40	50	60
26	50	50	50
27	50	50	50

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Table 2. Antiviral activity of non-toxic doses for tested compounds against Coxsackievirus B4.

Tested compounds	Initial viral titer	Final viral titer	% of reduction	Mean % of reduction
25	$1X10^5$	$4X10^4$	60%	56.7%
	$1X10^6$	$4X10^5$	60%	
	$1x10^7$	$5x10^6$	50%	
24	$1X10^5$	$1X10^4$	90%	90%
	$1X10^6$	$1X10^5$	90%	
	$1x10^7$	$1X10^6$	90%	
26	$1X10^5$	$4X10^4$	60%	56.00%
	$1X10^6$	$4X10^5$	60%	
	$1x10^7$	$5x10^6$	50%	
19	$1X10^5$	$4X10^4$	60%	53.3%
	$1X10^6$	$5X10^5$	50%	
	$1x10^7$	$5x10^6$	50%	

Table 3. Antiviral activity of non-toxic doses for the tested compounds against adenovirus type 7.

Tested compounds	Initial Viral titer	Final viral titer	% of reduction	Mean % of reduction
24	$1X10^5$	$5X10^4$	50%	50%
	$1X10^6$	$5X10^5$	50%	
	$1x10^7$	$5x10^6$	50%	
25	$1X10^5$	$4X10^4$	60%	58.00%
	$1X10^6$	$4X10^5$	60%	
	$1x10^7$	$5X10^6$	50%	

Table 4. Antiviral activity of nontoxic doses of tested compounds against rotavirus Wa strain.

Tested compounds	Initial Viral titer	Final viral titer	% of reduction	Mean % of reduction
26	$1X10^5$	$4X10^4$	60%	53.00%
	$1X10^6$	$5X10^5$	50%	
	$1x10^7$	$5x10^6$	50%	
24	$1X10^5$	$2X10^4$	80%	73.3%
	$1X10^6$	$3X10^5$	70%	
	$1x10^7$	$3X10^6$	70%	
25	$1X10^5$	$3X10^4$	70%	63.3%
	$1X10^6$	$3X10^5$	70%	
	$1x10^7$	$5x10^6$	50%	

All other compounds gave weak antiviral effect against tested viruses.

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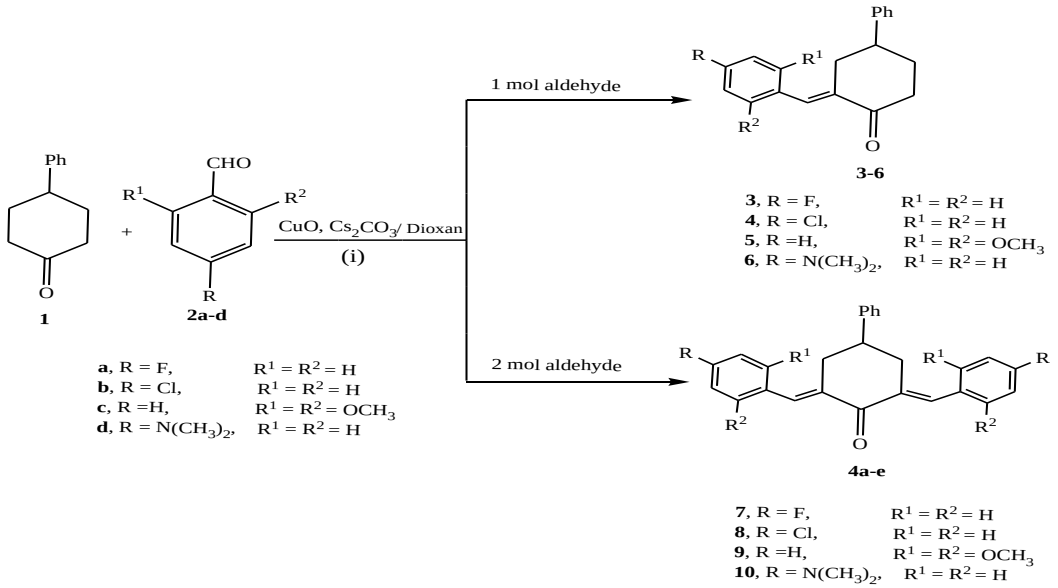
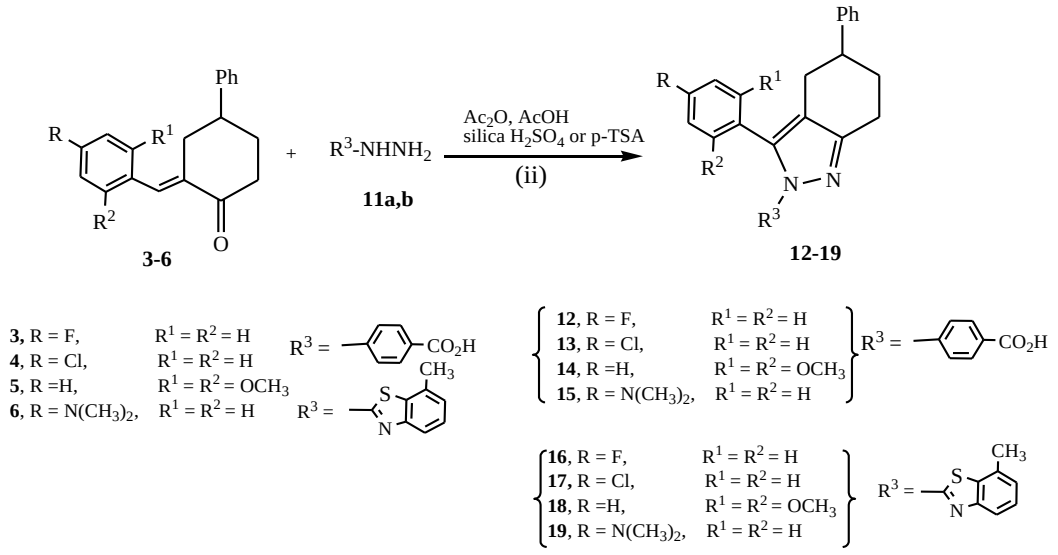


Scheme 1. Synthesis of phenylcyclohexanone derivatives (3-6) and benzylidene-4-phenylcyclohexanone derivatives (7-10). Reagents and conditions:(i) dioxane and DMF-DMA, cuprous oxide and Cs_2CO_3/reflux.



Scheme 2. Synthesis of tetrahydro-5-phenylindazol-2-yl)benzoic acid derivatives (12-15) and tetrahydro-2-(6-methylbenzothiazol-2-yl)-5-phenyl-2H-indazole derivatives (16-19). Reagents and conditions: (ii) acetic acid and acetic anhydride in the presence of silica sulfuric acid (5 mol %) /reflux or P-TSA/reflux.

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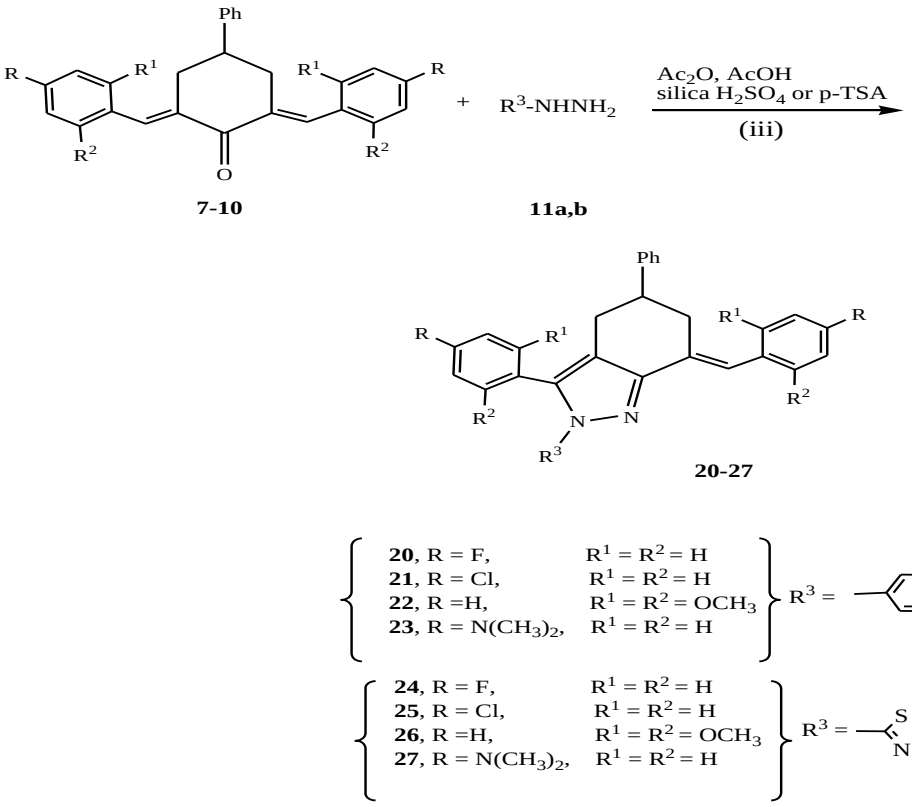

Scheme 3. Synthesis of benzylidene-tetrahydro-3-(4-phenyl)-5-phenylindazol-2-yl)benzoic acid derivatives (20-23) and tetrahydro-2-(6-methylbenzothiazol-2-yl)-5-phenyl-2H-indazole derivatives (24-27). Reagents and conditions: (iii) acetic acid and acetic anhydride in the presence of silica sulfuric acid (5 mol %) /reflux or P-TSA/reflux.

CONCLUSION

Viral gastroenteritis affects large numbers of children around the world. There is no available treatment for Coxsackievirus B4, adenovirus type 7 and rotavirus Wa strain. We herein synthesized new indazole compounds that were evaluated for their antiviral activity against Coxsackievirus B4, adenovirus type 7 and Wa strain viruses. This study develops a straightforward efficient approach to the synthesis of indazole derivatives with the incorporation of a cuprous oxide and Cs_2CO_3 as a heterogonous catalyst. The short reaction times of this procedure, high yields, and easy work up are some advantages of this method used for the preparation of 4,5,6,7-tetrahydro-5-phenylindazol-2-yl)benzoic acid derivatives and 4,5,6,7-tetrahydro-2-(6-methylbenzo[d]thiazol-2-yl)-5-phenyl-2H-indazolederivatives. The synthesized compounds were tested for their antiviral activity against Coxsackievirus B4, adenovirus type 7 and against rotavirus Wa strain. Compound24 exhibited moderate activity against Coxsackievirus B4 and rotavirus Wa strain. On the other hand compounds 24 and 25 showed promising activities against adenovirus type 7. Compounds 25 and 26 revealed promising activities against rotavirus Wa strain.

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