Enaminone as Building Blocks in Organic Chemistry: A Novel Route to Polyfunctionally 2-Substituted 5,6,7,8-Tetrahydronaphthalenes and Their Antiviral Evaluation

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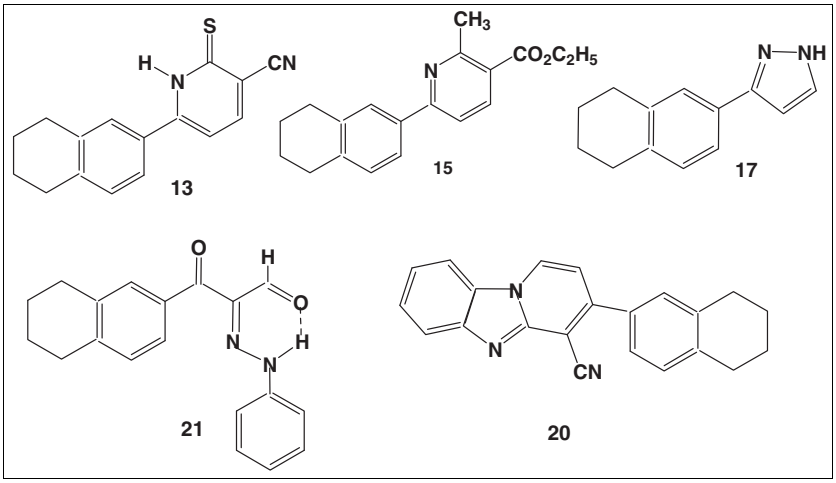

The enaminone **2** reacts with different reagents to afford anilino, aroylpyridine, 1,3,5-tri-tetrahydronaphthoylbenzene, pyridine, 2,3,6-trisubstituted pyridines, pyrazole, pyrido[1,2-*a*]benzimidazole, and hydrazone derivatives **4**, **6**, **8**, **9**, **11**, **13**, **15**, **17**, **20**, and **21**. The antiviral evaluation of some selected new products showed promising antiviral activity against human adenovirus 7 and human rotavirus Wa strain.

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INTRODUCTION

Enaminone derivatives are highly reactive intermediates and extensively received a considerable attention in the synthesis of a wide variety of biologically active heterocyclic systems [1–3]. Tetralines (tetrahydronaphthalene derivatives) are of increasing interest since many of these compounds play a vital role in the biological and pharmacological activities, because of their biological potentialities; for example, as potent agonists for D_2-type receptors [4], for the treatment of Alzheimer's disease [5], cardiovascular diseases [6], and as a preventer of dopamine-induced cell death [7]. On the other hand, the importance of the pyridine ring in the chemistry has been greatly realized because of its presence as a substructure in many natural products of therapeutically importance, involved in oxidation–reduction processes. The potent biological activity of various vitamins and drugs [8–11] is primarily contributed to the presence of a pyridine ring in their molecular make-up. In addition to various therapeutic applications, the importance of pyridines in the preparative organic chemistry cannot be ignored.

Therefore, in continuation to our previous interest [12–15] in the synthesis of a variety of heterocyclic systems from a readily obtainable inexpensive starting materials for biological screening program in our laboratory, we report here on the behavior of the versatile hitherto *E*-3-(*N,N*-dimethylamino)-1-(5,6,7,8-tetrahydronaphthalen-2-yl) prop-2-en-1-one **2** and its utility as a building block for the synthesis of the target compounds for antiviral screening.

Adenovirus 7 belongs to the Adenoviridae family. It contains a linear, double-stranded DNA about 30,000–42,000 nucleotides long. It has icosahedral morphology and no envelope. This virus spreads rapidly among people living in close proximity, such as military barracks, hospital wards, and chronic-care facilities. Adenovirus 7 causes acute respiratory disease, pharyngoconjunctival fever, conjunctivitis, and gastroenteritis.

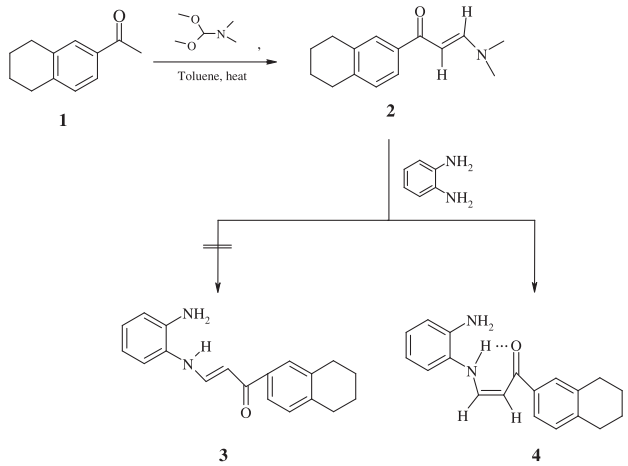
Rotaviruses have been detected in water, wastewater, and biosolids. These viruses are extremely resistant to environmental stress due to the presence of a double icosahedral shell. Rotavirus Wa strain was chosen as a representative example of the Reoviridae, because it is of human origin and is cultured on MA104 cell line. Rotaviruses are the first causes of gastroenteritis in children worldwide [16]. So, our goal is to find effective anti-adenovirus and rotavirus compounds.

RESULTS AND DISCUSSION

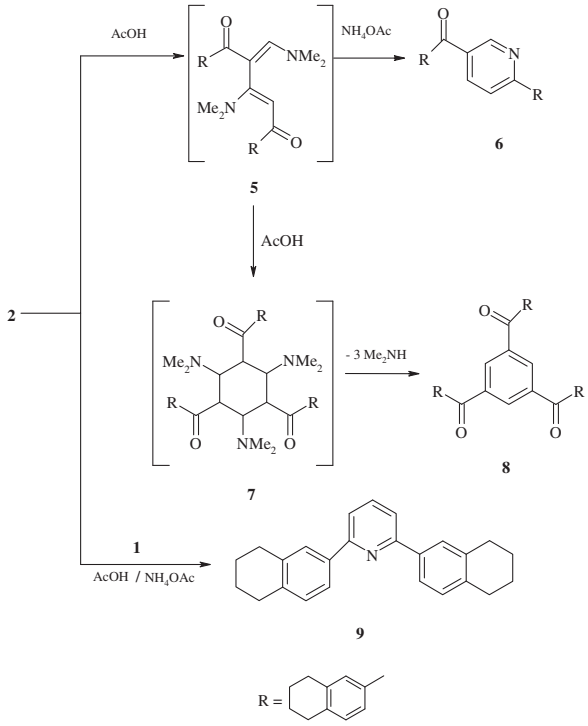
Chemistry. The versatile hitherto *E*-3-(*N,N*-dimethylamino)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-one (**2**) was prepared by the treatment of 2-acetyl tetralin (**1**) with dimethylformamide-dimethyl acetal (DMF-DMA) in refluxing dry toluene. The structure of the isolated product was confirmed by elemental analysis and spectral data. For example, its ^{1}H-NMR spectrum displayed two singlets at δ 2.87, 3.10 ppm due to *N,N*-dimethyl protons and two doublets at δ 5.76 and 7.65 ppm (J = 12.2 Hz) due to olefinic protons, in addition to an aromatic multiplet at the region δ 7.05–7.58 ppm and a multiplet at the region δ 1.71, 2.72 ppm for 4CH_2 tetrahydronaphthalene. The value of the coupling constant (J = 12.2 Hz) for the ethylenic protons indicates that the enaminones **2** exists all in the *E*-configuration. The reaction of **2** with *o*-phenylenediamine afforded products of condensation *via* elimination of dimethylamine. This was assigned *Z* structure **4** rather than *E* structure **3** based on ^{1}H-NMR spectrum, which revealed signals for *E* olefinic protons at δ 6.08 and δ 7.78 ppm (J = 7.5 Hz). The predominance of this form may be due to the fixation by hydrogen bonding (Scheme 1) [17].

Refluxing of compound **2** in acetic acid/ammonium acetate afforded the pyridine derivative **6** (Scheme 2). This was assumed to be formed *via* initial addition of two molecules of **2** yielding intermediate **5** that was cyclized by the action of ammonia into **6** [18]. In a trial to isolate the intermediate **5**, enaminone **2** was refluxed in acetic acid alone; however, under this condition the tri-tetrahydronaphthoylbenzene derivative **8** was the only isolable product. It is most likely that intermediate **5** reacted swiftly with a third molecule of **2** yielding the intermediate **7** that loses three molecules of dimethylamine yielding the final product **8**. The structures of compounds **6** and **8** were confirmed by ^{1}H-NMR, MS, and IR spectra. In addition, when enaminone **2** reacted with 2-acetyl tetralin (**1**) in acetic acid/ammonium acetate, afforded the pyridine derivative **9**, its structure was confirmed by ^{1}H-NMR, MS spectra. IR spectrum of the compound showed the disappearance of the carbonyl group (Scheme 2).

Scheme 1



Scheme 2



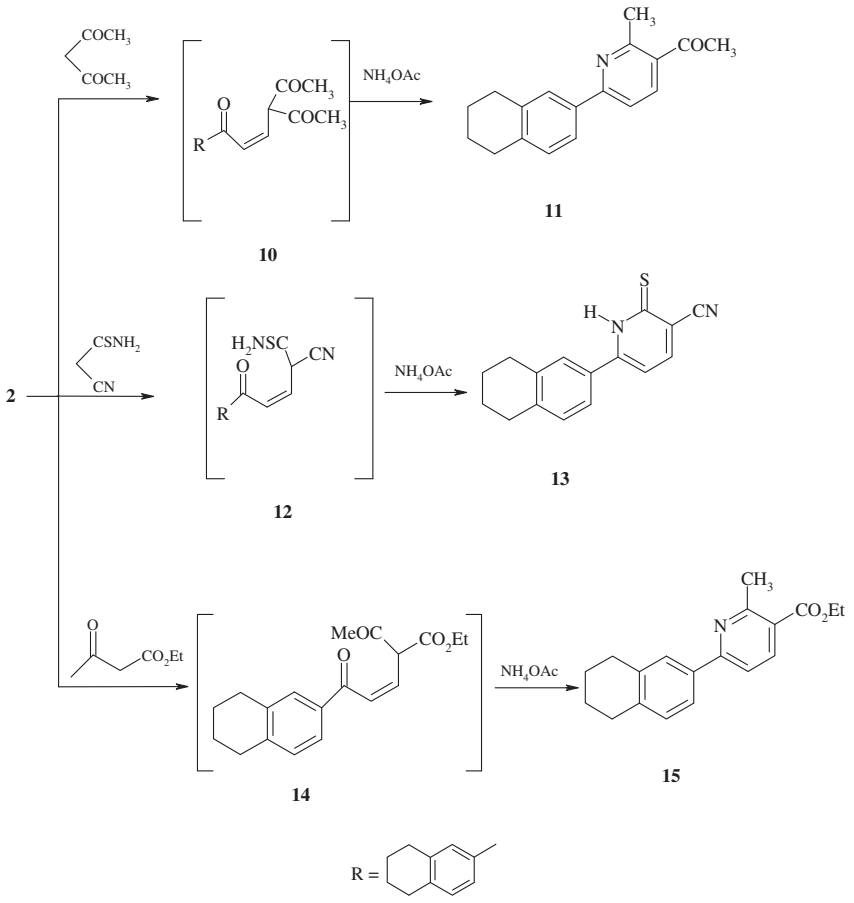
The reaction of compound **2** with acetyl acetone in refluxing acetic acid in the presence of ammonium acetate yielded the product that might be formulated as the pyridine derivative **11**, which was confirmed by ^{1}H-NMR, MS, and IR spectra. It is believed that **10** is an intermediate (Scheme 3).

Similar to the behavior of **2** toward acetyl acetone, it was reacted with cyanothioacetamide to yield **13** (Scheme 3). Structure of compound **13** is preferred over possible 4-tetrahydronaphthalene pyridine structures based on ^{1}H-NMR spectrum, which revealed pyridyl hydrogen resonances as doublets signals with J = 8.40 Hz. Isomeric 4-tetrahydronaphthalene pyridine should show lower coupling constants J = 4–6 Hz [19].

In addition, enaminone **2** was reacted smoothly with ethyl acetoacetate in refluxing acetic acid in the presence of ammonium acetate to yield the product that was formulated as pyridine derivative **15**, based on ^{1}H-NMR spectrum, which indicated pyridyl H-5 and H-4 as two doublets at δ 8.03 and 8.18 ppm with J = 8.4 Hz. If these were for H-3 and H-2, one would expect J = 4–6 Hz [19] (Scheme 3).

The reactivity of enaminone **2** toward some nitrogen nucleophiles was investigated. Thus, treatment of compound **2** with hydrazine hydrate, in refluxing ethanol, gave

Scheme 3



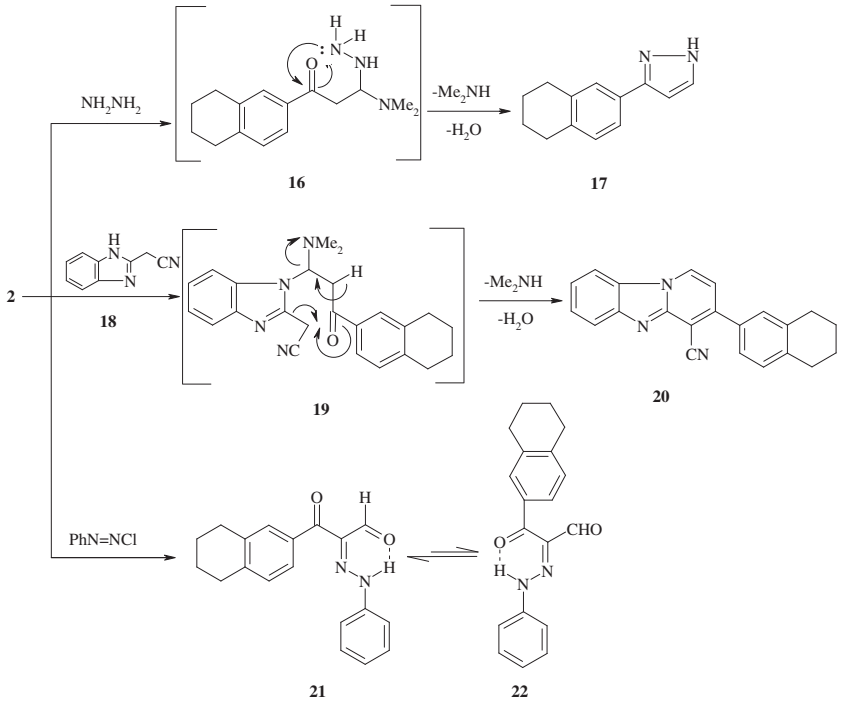
the product **17** (Scheme 4). The formation of **17** was assumed to take place *via* Michael-type addition of the amino group of hydrazines to the enamine double bond in compound **2** to form the nonisolable acyclic intermediate **16**, which readily undergoes intramolecular cyclization into the pyrazole derivative **17** *via* loss of dimethylamine and water molecules. The chemical structure of **17** was established on the basis of elemental analysis and spectral data of the isolated reaction product for example, the appearance of NH at 3218 cm^{-1} and disappearance of the carbonyl group in IR spectrum, also its ^{1}H-NMR spectrum revealed D_2O exchangeable signal at δ 12.76 ppm due to NH proton in addition to the presence of three doublet signals at δ 6.57, 7.02, 7.44 ppm and one singlet at δ 7.61 ppm due to pyrazol and aromatic protons.

On the other hand, heating equimolar amounts of compound **2** and benzimidazoleacetonitrile **18**, in ethanol in the presence of a catalytic amount of piperidine resulted in the formation of a single pure product **20** (as examined by TLC). The structure of the isolated product was identified as 3-(5,6,7,8-tetrahydronaphthalen-2-yl)-benz[4,5]imidazo [1,2-*a*]pyridine-4-carbonitrile (**20**) on the basis of its elemental analysis, IR, MS ,^{1}H–NMR, and ^{13}C-NMR spectral data. For example, IR spectrum of compound **20** showed a nitrile absorption band at 2227 cm^{-1}, whereas its ^{1}H-NMR spectrum displayed nine aromatic signals at the region δ 6.69–8.30 ppm.

Similar to the established behavior of enaminones towards aromatic diazonium salts [20, 21], enaminone **2** was coupled with benzene diazonium chloride to yield the phenylhydrazino derivative **21** as major reaction product [22, 23]. The chemical structure of compound **21** was established on the basis of its elemental analysis, IR, MS, ^{1}H-NMR, and ^{13}C-NMR spectral data. ^{1}H NMR revealed two signals at δ 9.54 and 9.98 ppm for a total of one proton, which indicated that the product exist in DMSO solution as mixtures of the anti form **21** and syn form **22**. The antiform generally predominated; this observation is also in accordance with that reported [24]. On the other hand, if there is no intramolecular hydrogen bonding, the aldehyde proton is expected at around 11 ppm according to Al-Matar *et al*. [22].

Antiviral screening. The nontoxic dose for all the samples was 0.1 mg/mL either on Hep2 or MA104 cell lines. Some samples did not show effect on either adenovirus 7 or human rotavirus Wa strain. Those samples are **4**, **6**, **8**, **9**, and **11**. Four samples showed antiviral effect on

Scheme 4



adenovirus type 7 and five samples showed antiviral effect on human rotavirus Wa strain ranged from 90 to 99% Tables 1 and 2. The CC_{50} ranged from 0.21 to 0.26 mg/mL on MA104 cell line and ranged from 0.23 to 0.28 mg/mL on Hep 2 cell line Tables 3 and 4.

It obvious that the best antiviral activity against adenovirus 7 and human rotavirus Wa strain was gained by the compounds **13**, **15**, **17**, **20**, and **21**. These results can be returned to the attachment of the respective thioxopyridine, pyridine, pyrazole benzimidazo pyridine, and/or phenylhydrazino ring systems to tetralin nucleus. These moieties may either affect the viral DNA or viral protein. In addition, the inhibition of viral adsorption to the host cells or interruptions of the viral life cycles are all other possibilities. In our screening for antiviral activity of the tested compounds, it has been noted that compound **17** did not show any effect on adenovirus 7, and this may be returned to that compound **17** could affect only the RNA of rotavirus.

CONCLUSION

Some tested compounds **13**, **15**, **17**, **20**, **21** showed antiviral effect against adenovirus 7 and human rotavirus Wa strain, which may be promising compounds as anti-adenovirus 7 and human rotaviruses.

Table 1

Minimal nontoxic dose and anti-rotavirus Wa strain activity of *tested* materials on MA104 cells determined by the end-point titration technique.

Tested materials	Viral reduction factor
4	1
6	1
8	1
9	1
11	1
17	10
13	10
15	10
20	10^2
21	10^2

Table 2

Minimal nontoxic dose and anti-adenovirus type 7 activity of tested materials on Hep2 cells determined by the end-point titration technique.

Tested materials	Viral reduction factor
4	1
6	1
8	1
9	1
11	1
17	1
13	10
15	10
20	10
21	10

Table 3

Anti-rotavirus Wa strain activity of tested materials on MA104 Cells determined by the MTT method.

Tested materials	CC_{50} (mg/mL)	IC50 (mg/mL)	Therapeutic index
4	0.23	ND	ND
6	0.21	ND	ND
8	0.26	ND	ND
9	0.24	ND	ND
11	0.24	ND	ND
17	0.23	0.053	ND
13	0.21	0.057	4.21
15	0.25	0.051	4.9
20	0.22	0.047	4.68
21	0.23	0.045	5.11

ND: not done.

Table 4

Anti-adenovirus type 7 strain activities of tested materials on Hep2 cells determined by the MTT method.

Tested materials	CC_{50} (mg/mL)	IC50 (mg/mL)	Therapeutic index
4	0.26	ND	ND
6	0.24	ND	ND
8	0.28	ND	ND
9	0.26	ND	ND
11	0.27	ND	ND
17	0.24	ND	ND
13	0.23	0.059	3.89
15	0.27	0.056	4.82
20	0.24	0.054	4.44
21	0.25	0.056	4.46

ND: not done.

EXPERIMENTAL

Chemistry. All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were performed by Vario El-Mentar apparatus (Shimadzu, Japan), National Research Centre, Cairo, Egypt. IR spectra were recorded on a Biorad FTS 155 FT-IR spectrophotometer (ICB-IR Service Centre, Pozzuoli, Naples, Italy) or recorded as potassium bromide pellets on a Perkin-Elmer 1650 Spectrophotometer, National Research Centre, Cairo, Egypt. ^{1}H-NMR experiments were conducted at ICB-NMR Service Centre (Pozzuoli, Naples, Italy) and were acquired in DMSO, (shifts are referenced to the solvent signal) on a Bruker Avance-400 operating at 400 MHz, and/or ^{1}H-NMR spectra were determined in DMSO-d_6 at 300 MHz (^{1}H-NMR) and at 75 MHz (^{13}C-NMR) on a Varian Mercury VX 300 NMR spectrometer using TMS as an internal standard (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were carried out on an ion-trap MS instrument in EI mode at 70 ev (ICB-IR Service Centre, Pozzuoli, Naples, Italy) or determined on Shimadzu GCMS-QP-1000EX mass spectrometer at 70 ev (Cairo University, Cairo, Egypt).

Compounds **1** and **18** were prepared according to the reported methods [25,26].

E-3-(N,N-Dimethylamino)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-one (2). To a mixture of 2-acetyl tetralin (**1**; 1.74 g, 10 mmol) in dry toluene (50 mL), DMF-DMA (1.34 g, 10 mmol) was added, and the mixture was refluxed for 5 h. The solvent was evaporated, and the residual reddish brown viscous liquid was taken in ether. The resulting yellow crystals were collected by filtration, washed thoroughly with ether, dried and finally recrystallized from EtOH to afford compound **2** as yellow crystals in 58% yield, mp 70–72°C; IR (KBr) (v, cm^{-1}): 1634 (C=O); ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.71 (m, 4H, aliphatic $2CH_2$ of tetrahydronaphthalene), 2.72 (m, 4H, aliphatic $2CH_2$ of tetrahydronaphthalene), 2.87 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 5.76 (d, 1H, J = 12.2 Hz, -CO-CH=), 7.05–7.58 (m, 3H Ar-H), 7.65 (d, 1H, J = 12.2 Hz, =CH-N-); ^{13}C-NMR (DMSO-d_6): δ (ppm) 20.12, 20.13 ($2CH_3$), 22.48, 22.597, 28.70, 28.75 ($4CH_2$), 90.93 (CO-CH=), 124.25, 127.67, 128.44, 136.17, 137.52, 139.62 (aromatic-C), 153.63 (=CH-N-), 185.70 (C=O); MS m/z (%): 229 (M$^+$, 92.4), 212 (99.0), 159 (43.5), 98 (100), 70 (74.5). Anal. calcd for $C_{15}H_{19}NO$ (229.32): required C, 78.56; H, 8.35; N, 6.11; found C, 78.24; H, 8.03; N, 6.28.

(Z)-3-(2-Aminophenylamino)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)-prop-2-en-1-one (4). A mixture of enaminone **2** (2.29 g, 0.01 mol) and *o*-phenylenediamine (1.08 g, 0.01 mol) in dry ethanol (30 mL) was refluxed for 8 h. The solvent was partially removed, and the obtained precipitate was filtered off and recrystallized from ethanol to give compound **4**.

Yield (83%); m.p. 148–150°C; IR spectrum (KBr, v, cm^{-1}): 3381, 3331, 3132 (NH_2, NH), 2925 (CH alicyclic), 1640 (CO); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.75 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.77 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 4.81 (d, 2H, NH_2, exchangeable with D_2O), 6.08 (d, J = 7.50 Hz, 1H, CH=), 6.61–7.74 (m, 7H, Ar-H), 7.79 (d, J = 7.50 Hz, 1H, CH=), 11.96 (br, 1H, NH, D_2O exchangeable); MS *m/z* (%): 292 (M$^+$, 43), 291 (M$^+$-1, 62), 159 (80) and 119 (100); Anal. calcd for $C_{19}H_{20}N_2O$ (292.38): required C, 78.05; H, 6.89; N, 9.58; found C, 78.28; H, 6.87; N, 9.57.

(5,6,7,8-Tetrahydronaphthalen-2-yl)-6-[(5,6,7,8-tetrahydronaphthalen-2-yl)-pyridin-3-yl)]-methanone (6). A mixture of enaminone **2** (2.29 g, 0.01 mol) and ammonium acetate (0.77g, 0.01 mol) was refluxed in glacial acetic acid (20 mL) for 1h, then left to cool. The separated solid was filtered off and recrystallized from ethanol-DMF to give compound **6**.

Yield (81%); m.p. 132–134°C; IR spectrum (Biorad FTS, v, cm^{-1}): 2930 (CH alicyclic), 1651 (CO), 1587 (C=N); ^{1}H-NMR (DMSO-d_6, δ ppm) : 1.77 (m, 8H, $4CH_2$ of tetrahydronaphthalene), 2.79 (m, 8H, $4CH_2$ of tetrahydronaphthalene), 7.19–7.26 (m, 2H, Ar-H), 7.53 (d, J = 13.50 Hz, 2H, Ar-H), 7.87 (d, J = 9.22 Hz, 2H, Ar-H), 8.06–8.19 (m, 2H, Ar-H,), 9.07 (s, 1H, pyridyl H-2); MS *m/z* (%): 368 (M$^+$+1, 100), 367 (M$^+$, 30); Anal. calcd for $C_{26}H_{25}NO$ (367.50): required C, 84.98; H, 6.86; N, 3.81; found C, 84.96; H, 6.54; N, 3.95.

1,3,5-Tri-2-(5,6,7,8-Tetrahydronaphtholyl)benzene (8). Compound **2** (2.29 g, 0.01 mol) was refluxed in glacial acetic acid (20 mL) for 4 h, then left to cool. The separated solid was filtered off and recrystallized from glacial acetic acid to afford compound **8**.

Yield (89%); m.p. 158–160°C; IR spectrum (Biorad FTS, v, cm^{-1}): 2931 (CH alicyclic), 1659 (3CO), 1604 (C=C); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.75 (m, 12H, 6 CH_2 of tetrahydronaphthalene), 2.78 (m, 12H, $6CH_2$ of tetrahydronaphthalene), 7.25 (d, J = 7.60 Hz, 3H, Ar-H), 7.53 (s, 3H, Ar-H), 7.55 (s, 3H, Ar-H), 8.18 (d, J = 9.0 Hz, 3H, Ar-H); MS *m/z* (%): 552 (M$^+$, 8), 159 (100); Anal. calcd for $C_{39}H_{36}O_3$ (552.72): required C, 84.75; H, 6.57; found C, 84.87; H, 6.55.

2,6-Bis-(5,6,7,8-tetrahydronaphthalen-2-yl)-pyridine (9). A mixture of compound **2** (2.29 g, 0.01 mol), acetyl tetralin **1** (1.74 g, 0.01 mol), and ammonium acetate (0.77 g, 0.01 mol) was refluxed in glacial acetic acid (30 mL) for 1 h. The precipitated solid was isolated by filtration and recrystallized from ethanol to afford compound **9**.

Yield (66%); m.p. 140–142°C; IR spectrum (Biorad FTS, v, cm^{-1}): 2936 (CH alicyclic), 1590 (C═N); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.75 (m, 8H, $4CH_2$ of tetrahydronaphthalene), 2.77 (m, 8H, $4CH_2$ of tetrahydronaphthalene), 7.16–8.14 (m, 9H, Ar-H); MS *m/z* (%): 339 (M^+, 20), 208 (37), 159 (100); Anal. calcd for $C_{25}H_{25}N$ (339.48): required C, 88.45; H, 7.42; N, 4.13; found C, 88.21; H, 7.44; N, 4.35.

1-[2-Methyl-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-pyridin-3-yl]-ethanone (11). To a solution of enaminone **2** (2.29 g, 0.01 mol) and ammonium acetate (1 g, 0.01 mol) in glacial acetic acid (30 mL), acetyl acetone (1 g, 0.01 mol) was added, and the reaction mixture was refluxed for 2 h, cooled, poured onto ice-water. The separated solid was filtered off and recrystallized from ethanol to afford compound **11**.

Yield (81%); m.p. 104–106°C; IR spectrum (KBr, v, cm^{-1}): 2926 (CH alicyclic), 1646 (CO), 1581 (C═N); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.78 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.59 (s, 3H, $COCH_3$), 2.69 (s, 3H, CH_3), 2.80 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 7.21 (d, *J* = 7.80 Hz, 1H, Ar-H), 7.51 (s, 1H, Ar-H), 7.87 (d, *J* = 7.50 Hz, 1H, Ar-H), 8.09 (d, *J* = 8.10 Hz, 1H, pyridyl H-5), 8.91 (d, *J* = 8.10 Hz, 1H, pyridyl H-4); MS *m/z* (%): 265 (M^+, 86), 250 (100); Anal. calcd for $C_{18}H_{19}NO$ (265.36): required C, 81.48; H, 7.22; N, 5.28; found C, 81.34; H, 7.41; N, 5.13.

(5,6,7,8-Tetrahydronaphthalen-2-yl)-2-thioxohydropyridine-3-carbonitrile (13). To a stirred suspension of **2** (2.29 g, 0.01 mol) and ammonium acetate (1 g, 0.01 mol) in glacial acetic acid (10 mL), cyanothioacetamide (1 g, 0.01 mol) was added, and the reaction mixture was refluxed for 1 h then allowed to cool. The separated solid was filtered off and recrystallized from ethanol to afford compound **13**. Yield (73%); m.p. 202–204°C; IR spectrum (KBr, v, cm^{-1}): 3193 (NH), 2928 (CH alicyclic), 2219 (CN); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.75 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.77 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 7.19 (d, *J* = 7.20 Hz, 1H, Ar-H), 7.49 (d, *J* = 7.20 Hz, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 8.19 (d, *J* = 8.40 Hz, 1H, pyridyl H-5), 8.59 (d, *J* = 8.40 Hz, 1H, pyridyl H-4), 12.00 (s, 1H, NH, exchangeable with D_2O); MS *m/z* (%): 266 (M^+, 100); Anal. calcd for $C_{16}H_{14}N_2S$ (266.37): required C, 72.15; H, 5.30; N, 10.52; S, 12.03; found C, 72.14; H, 5.34; N, 10.49; S, 12.23.

2-Methyl-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-nicotinic acid ethyl ester (15). To a solution of enaminone **2** (0.573 g, 0.025 mol) and ammonium acetate (0.385 g, 0.005 mol) in glacial acetic acid (10 mL), ethyl acetoacetate (0.325 g, 0.025 mol) was added, and the reaction mixture was refluxed for 1 h, then the solvent was evaporated under reduced pressure, and the residue was recrystallized from ethanol to give compound **15**.

Yield (88%); m.p. 92–94°C; IR spectrum (Biorad FTS, v, cm^{-1}): 2924 (CH alicyclic), 1719 (CO), 1586 (C═N); ^{1}H-NMR (DMSO-d_6, δ ppm) : 1.32 (t, 3H, *J* = 7.2 Hz, CH_3), 1.74 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.73 (s, 3H, CH_3), 2.77 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 4.31 (q, 2H, OCH_2), 7.23 (d, *J* = 7.4 Hz, 1H,), 7.81–7.87 (m, 2H, Ar-H), 8.03 (d, *J* = 8.40 Hz, 1H, pyridyl H-5), 8.18 (d, *J* = 8.40 Hz, 1H, pyridyl H-4); MS *m/z* (%): 295 (M+, 40), 225 (100); Anal. calcd for $C_{19}H_{21}NO_2$ (295.38): required C, 77.26; H, 7.17; N, 4.74; found C, 77.49; H, 7.15; N, 4.42.

3-(5,6,7,8-Tetrahydronaphthalen-2-yl)-1H-pyrazole (17). To a solution of enaminone **2** (2.29 g, 0.01 mol) in dry ethanol (30 mL), hydrazine hydrate (2 mL, 80%) was added, and the reaction mixture was refluxed for 4 h. The separated solid after cooling was filtered off and recrystallized from ethanol to afford compound **17**.

Yield (69%); m.p. 62–64°C; IR spectrum (KBr, v, cm^{-1}): 2925 (CH alicyclic), 3218 (NH); ^{1}H-NMR spectrum (DMSO-d_6, δ ppm): 1.75 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.75 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 6.57 (d, *J* = 2.3 Hz, 1H, pyrazole-4-CH), 7.02 (d, *J* = 7.65 Hz, 1H, Ar-H), 7.44 (d, *J* = 7.65, 2H, Ar-H+ pyrazole 5-CH), 7.61 (s, 1H, Ar-H), 12.76 (s, 1H, NH, exchangeable with D_2O); MS *m/z* (%): 198 (M^+, 73), 170 (100); Anal. calcd for $C_{13}H_{14}N_2$ (198.27): required C, 78.75; H, 7.12; N, 14.13; found C, 78.61; H, 7.22; N, 14.42.

3-(5,6,7,8-Tetrahydronaphthalen-2-yl)-benz[4,5]imidazo[1,2-a]pyridine-4-carbonitrile (20). To a mixture of enaminone **2** (0.573 g, 0.025 mol) and 1H-benzimidazole-2-acetonitrile **18** (0.393 gm, 0.025 mol) in dry ethanol (30 mL), piperidine (0.3 mL) was added, and the reaction mixture was refluxed for 12 h. The precipitated product was filtered off, washed with ethanol dried. Recrystallized from DMF afforded compound **20**.

Yield (83%); m.p. 236–238°C; IR spectrum (KBr, v, cm^{-1}): 2925 (CH alicyclic), 2227 (CN), 1593 (C═N); ^{1}H-NMR (DMSO-d_6, δ ppm) : 1.84 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.88 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 6.69 (d, *J* = 8.40 Hz, 1H, Ar-H), 6.91 (d, *J* = 7.50 Hz, 1H, Ar-H), 7.11–7.53 (m, 5H, Ar-H), 7.91 (d, *J* = 8.40 Hz, 1H, Ar-H), 8.30 (d, *J* = 7.20 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ (ppm): 22.41, 22.51, 28.67, 28.72 ($4CH_2$), 119.61 (CN), 111.41, 114.65, 115.77, 121.30, 125.29, 126.01, 128.70, 129.57, 130.02, 137.81, 137.84, 139.69, 144.28, 146.30, 146.50 (Aromatic-C). MS *m/z* (%): 323 (M^+, 100); Anal. calcd for $C_{22}H_{17}N_3$ (323.40): required C, 81.71; H, 5.30; N, 12.99; found C, 81.52; H, 5.45; N, 13.97.

3-Oxo-2-(phenylhydrazino)-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-propionaldehyde (21). A cold solution of benzene diazonium salt (0.01 mol) was prepared by a solution of sodium nitrite (0.01 mol in 3 mL water) to a cold solution of aniline with stirring. The resulting solution of benzene diazonium salt was added to a cold solution of enaminone **2** (2.29 g, 0.01 mol) in dry ethanol (50 mL) containing sodium acetate. The reaction mixture was stirred for 30 min at room temperature and the formed solid was filtered off, washed with water, and recrystallized from ethanol to give compound **21**.

Yield (76%); m.p. 82–84°C; IR spectrum (KBr, v, cm^{-1}): 2921 (CH alicyclic), 3130 (NH) 1646 (CH═O), 1631 (CO); ^{1}H-NMR spectrum (DMSO-d_6, δ ppm): 1.74 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.76 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 7.09–7.62 (m, 8H, Ar-H), 9.54,9.98 (s, 1H, CHO), 11.96,14.20 (s, 1H, NH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6): δ (ppm): 22.37, 22.53, 28.77, 28.88 ($4CH_2$), 116.65, 123.30, 125.71, 126.13, 127.31, 128.26, 129.19, 130.88, 137.81, 137.84, 138.53, 141.42 (Aromatic-C), 143.85 (C═N), 188.28, 188.46, 189.99, 191.91 (CO). MS *m/z* (%): 306 (M^+, 85), 186 (100%); Anal. Calcd. for $C_{19}H_{18}N_2O_2$ (306.37): required C, 74.49; H, 5.92; N, 9.14; found C, 74.21; H, 5.99; N, 9.35.

Cytotoxicity assay. All samples (100 mg) were dissolved in 500 μL of ethanol or acetic acid. Cell monolayers Hep2 and MA104 (obtained from The Holding Company for Biological Products & Vaccines VACSERA, Egypt) were trypsinized, washed with culture medium and plated in a 96-well flat bottomed plate with 5×10^3 cells per well for both cell lines. After 24 h incubation, each diluted (Greiner-Bio one, Germany) tested materials (10-fold dilutions of decontaminated samples

which 12 μL of 100x of antibiotic, antimycotic mixture was added to 500 μL of each sample) was added to the appropriate wells, and the plates were incubated for a further 48 h at 37°C in a humidified incubator with 5% CO_2. The supernatants were removed from the wells, and cell viability was evaluated using microscopic examination trypan blue and using the MTT technique [27–29]. The results are obtained from triplicate assays with at least five extract concentrations. The percentage of cytotoxicity is calculated as $[(A - B)/A] \times 100$, where A and B are the OD492 of untreated and of treated cells, respectively.

Antiviral testing. The *in vitro* antiviral screening method was used to estimate the inhibition of the cytopathic effect (CPE) of the pure compounds on MA104 and HEP-2 cell monolayers infected with rotavirus Wa strain (ATCC VR-2018, obtained by Prof. Dr. Albert Bosch, University of Barcelona, Spain) and adenovirus type 7 (obtained by Dr. Ali Fahmy, VACSERA, EGYPT) using the end-point titration technique (EPTT) [30]. Confluent monolayers of MA104 and HEP-2 cells were grown in 96-well microtiter plates, which were infected with serial 10-fold dilutions of a rotavirus Wa strain and adenovirus type 7 suspensions, respectively. The viruses were allowed to adsorb for 60 min at 37°C, after which serial twofold dilutions of the test compounds in maintenance medium, supplemented with 2% serum and antibiotics, were added. The plates were incubated at 37°C, and the viral cytopathic effect was recorded by light microscopy after 2–8 days. Virus suspensions are characterized by their virus titers, which are expressed as the smallest amount of virus capable of producing a reaction in the host cells. The antiviral activity is expressed as a reduction factor (RF), being the ratio of the viral titers in the virus control and in the presence of the maximal nontoxic dose of test substance.

MTT assay (antiviral colorimetric assay). Both MA104 and Hep2 cell monolayers were grown in 96-well microtiter plates. Dilutions of the extracts, prepared as described above for the EPTT assay, were added 1 h before viral infection. Ten infectious doses of virus were added to each well and incubated at 37°C in humidified 5% CO_2 atmosphere for 48 h. Controls consisted of untreated infected, treated uninfected, and untreated uninfected cells. Cell viability was evaluated by the MTT colorimetric technique [29]. Briefly, the supernatants were removed from the wells and 28 μL of an MTT (Sigma) solution (2 mg/mL in PBS) was added to each well. The plates were incubated for 1.5 h at 37°C, and 130 μL of DMSO was added to the wells to dissolve the MTT crystals. The plates were placed on a shaker for 15 min and the optical density was determined at 492 nm (OD492) on a multiwell spectrophotometer. The 50% cytotoxic concentration (CC50) of the test extract is defined as the concentration that reduce the OD492 of treated uninfected cells to 50% of that of untreated uninfected cells. The 50% antiviral effective concentration, that is, 50% inhibitory concentration of the viral effect (IC50) is expressed as the concentration that achieves 50% protection of treated infected cells from HSV-2 induced destruction. The percent protection is calculated as $[(A - B)/C - B)] \times 100$, where A, B, and C are the OD492 of treated infected, untreated infected, and untreated uninfected cells, respectively.

Data analysis. CC50 and IC50 for each compound were obtained from dose-effect-curves. The CC50 and IC50 are the average of four assays with five concentrations within the inhibitory range of the compounds. The therapeutic index (i.e., selective index) is defined as CC50/IC50.

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REFERENCES AND NOTES

[1] Greenhill, J. V. Chem Soc Rev 1977, 6, 277.
[2] Bejan, E.; Haddou, H. A.; Daran, J. C.; Balavoine, G. G. A. Synthesis 1996, 8, 1012.
[3] Stanovnik, B.; Steve, J. Chem Rev 2004, 104, 2433.
[4] Dutta, A. K.; Fei, X. S.; Reith, M. E. A. Bioorg Med Chem Lett 2002, 12, 619.
[5] Toddei, P; Torreggiani, A.; Fini, G. Biopolymers 2002, 67, 289.
[6] Hatori, H.; Sato, B.; Sato, I.; Shibata, T.; Tsurumi, Y.; Sakamoto, K.; Takase, S.; Veda, H.; Hino, M.; Fujii, T. J. Antibiotic 2004, 57, 264.
[7] Song, J. H.; Slot, A. J.; Ryan, R. W. J.; Ross, G. M. Neuropharmacology 2004, 46, 984.
[8] Joule, J. A.; Smith, G.; Mills, K. Heterocyclic Chemistry, 3rd ed.; Chapman and Hall: London, 1995, p 72.
[9] Roth, H. J.; Kleeman, A. Pharmaceutical Chemistry, Drug Synthesis; Prentice Hall Europe: London, 1988; Vol. 1, p 407.
[10] Henry, G. D. Tetrahedron 2004, 60, 6043.
[11] (a) Li, A. H.; Moro, S.; Forsyth, N.; Melman, N.; Ji, X. D.; Jacobsen, K. A. J Med Chem 1999, 42, 706; (b) Vacher, B.; Bonnaud, B.; Funes, F.; Jubaudt, N.; Koek, W.; Assie, M. B.; Cosi, C.; Kleven, M. J Med Chem 1999, 42, 1648; (c) Song, Z. S.; Zhao, M.; Demond, R.; Devine, P.; Tschaen, D. M.; Tillyer, R.; Frey, L.; Heid, R.; Xu, F.; Foster, B.; Li, J.; Reamer, R.; Volante, R.; Grabowski, E. J.; Dolling, U. H.; Reider, P. J. J Org Chem 1999, 64, 9658.
[12] Hamdy, N. A.; Gamal-Eldeen, A. M. Eur J Med Chem 2009, 44, 4547.
[13] Abdel-Aziz, H. A.; Gamal-Eldeen, A. M.; Hamdy, N. A.; Fakhr, I. M. I. Arch Pharm 2009, 342, 230.
[14] Hamdy, N. A.; Gamal-Eldeen, A. M.; Abdel-Aziz, H. A.; Fakhr, I. M. I. Eur J Med Chem 2010, 45, 463.
[15] Abdel-Aziz, H. A.; Hamdy, N. A.; Gamal-Eldeen, A. M.; Fakhr, I. M. I. Z Naturforsch 2011, 66c, 7.
[16] Chapron, C. D.; Ballester, N. A.; Fontaine, J. H.; Frades, C. N.; Margolin, A. B. Appl Environ Microbiol 2000, 66, 2520.
[17] Hassanien, A. A.; Ghozlan, S. A. S.; Elnagdi M. H. J Heterocyclic Chem 2003, 40, 225.
[18] Graf, E.; Troschutes, R. Synthesis 1999, 117, 1216.
[19] (a) Al-Moussawi, S.; Kaul, K.; Mohamed, M. A.; Elnagdi, M. H. J Chem Res 1997, S, 318; (b) Al-Moussawi, S.; Kaul, K.; Mohamed, M. A.; Elnagdi, M. H. 1997, M, 2026.
[20] Al-Omran, F.; Abdel-Khalik, M. M.; Elnagdi, M. H. Synthesis 1997, 91.
[21] Al-Awadi, H. A.; Ibrahim, Y. A.; Elnagdi, M. H. Tetrahedron 2001, 57, 1609.
[22] Al-Matar, H. M.; Riyadh, S. M.; Elnagdi, M. H. J Heterocyclic Chem 2007, 44, 603.
[23] El-Dusouqui, O. M. E.; Abdel-Khalik, M. M.; Al-Awadi, N. A.; Dib, H. H.; George, B. J.; Elnagdi, M. H. J Chem Res 2006, S, 295.
[24] Al-Shiekh, M. A.; El-Din, A. M. S.; Hafez, E. A.; Elnagdi, M. H. J Heterocyclic Chem 2004, 41, 647.
[25] Allinger, N. L.; Jones, E. S. J Org Chem 1962, 27, 70.
[26] Copeland, R. A. B.; Day, A. R. J Am Chem Soc 1943, 65, 1072.
[27] Simões, C.; Amoros, M.; Girre, L. Phytoth Res 1999, 21, 317.
[28] Walum, E.; Strenberg, K.; Jenssen, D. Principles and Pratice; Ellis Howood: New York, 1990; p 97.
[29] Mosmann, T.; Immuno, J. Methods 1983, 65, 55.
[30] Vlietinck, A. J.; Van Hoof, L.; Totté, J.; Lasure, A.; Vanden Berghe, D.; Rwangabo, P. C.; Mvukiyumwami, J. J Ethnopharmacol 1995, 46, 31.